(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 June 2003 (19.06.2003)

PCT

(10) International Publication Number
WO 03/050109 A1

(51) International Patent Classification[7]: C07D 401/12, 401/14, 401/04, 409/12, 409/14, 209/04, 333/52, A61K 31/38, 31/404, 31/445

(21) International Application Number: PCT/US02/37595

(22) International Filing Date: 9 December 2002 (09.12.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/339,325 12 December 2001 (12.12.2001) US

(71) Applicant *(for all designated States except US)*: **ELI LILLY AND COMPANY** [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **JONES, Stuart, Donald** [GB/GB]; 17 Oakwood Drive, Prestbury, Macclesfield, Cheshire SK10 4HG (GB). **SALL, Daniel, Jon** [US/US]; 3601 Ostrom Court, Greenwood, IN 46143 (US). **WILEY, Michael, Robert** [US/US]; 7725 Langwood Drive, Indianapolis, IN 46268 (US).

(74) Agents: **HAY, Martin, Alexander** et al.; Martin A. Hay & Co., 13 Queen Victoria Street, Macclesfield, Cheshire SK11 6LP (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

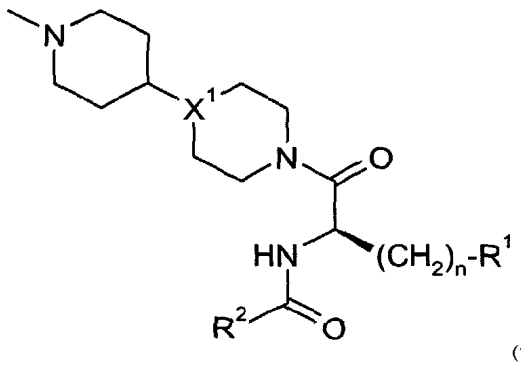
Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/050109 A1

(54) Title: ALANYL-PIPERIDINE HETEROCYCLIC DERIVATIVES USEFUL AGAINST CARDIOVASCULAR DISEASES



(1)

(57) Abstract: Compounds of formula (1) in which R^1, R^2, n and X^1 have the meanings given in the specification are Factor Xa inhibitors useful in the treatment of thrombotic disorders.

ALANYL-PIPERIDINE HETEROCYCLIC DERIVATIVES USEFUL AGAINST CARDIOVASCULAR DISEASES

The present invention relates to compounds useful as pharmaceuticals, to pharmaceutical compositions comprising the compounds, to a process for preparing the compounds, to intermediates useful in the preparation of the compounds, and to use of the compounds as pharmaceuticals.

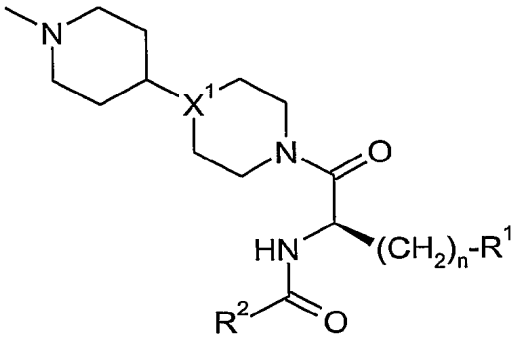
Cardiovascular disease continues to present a major worldwide health problem, and is a common cause of serious illness and death.

One line of investigation being pursued by researchers in the search for new treatments for cardiovascular disease is based upon the hypothesis that an inhibitor of the serine protease, Factor Xa, may be useful as an anticoagulant agent in the treatment of thrombotic disease.

Inhibitors of Factor Xa are known. For example, WO 99/11657, WO 99/11658 and WO 00/76971 disclose certain compounds containing an aromatic group, a glycine residue that bears a cyclic group and a lipophilic group. WO 99/11657, which discloses compounds in which the aromatic group is an aminoisoquinoline group, also generically discloses aminoisoquinoline compounds containing a glycine residue that bears an acyclic group.

Surprisingly, compounds containing particular phenyl, indolyl or benzo[b]thiophenyl groups, a glycine residue bearing a substituted alkyl group and a 4-(1-methyl-piperidin-4-yl)piperidin-1-yl or 4-(1-methylpiperidin-4-yl)piperazin-1-yl group have now been found that are selective Factor Xa inhibitors and have particularly advantageous properties.

Accordingly, the present invention provides a compound of formula (I)



(I)

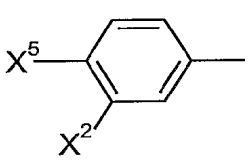
in which

X^1 represents CH or N;

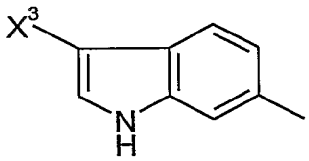
n is 1 or 2;

R^1 represents trifluoromethyl, COOH, $CONH_2$, SO_2NH_2, phenyl, pyridyl, C-linked imidazolyl (which may bear an N-(1-4C)alkyl substituent) or a (3-6C)cycloalkyl, oxa(4-6C)cycloalkyl, thia(4-6C)cycloalkyl or C-linked aza(4-6C)cycloalkyl group, which C-linked aza(4-6C)cycloalkyl group may bear an N-(1-4C)alkyl substituent; and

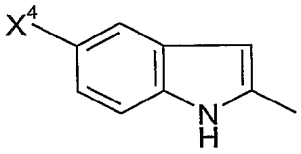
R^2 is selected from



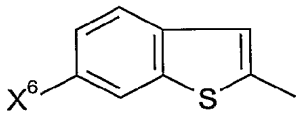
;



;



and



in which

X^2 represents a hydrogen atom, a halogen atom or an amino group;

X^3 represents a hydrogen atom, a methyl group, a fluorine atom, a chlorine atom or a bromine atom;

X^4 represents a hydrogen atom, a methyl group or a halogen atom;

X^5 represents a chlorine atom, a methoxy group or a methyl group; and

X^6 represents a hydrogen atom, a halogen atom or a methyl group;

or a pharmaceutically acceptable metabolically labile ester thereof, or a pharmaceutically acceptable salt thereof.

Compounds of formula (I) have been found to be potent and selective inhibitors of the serine protease, Factor Xa, to have good anticoagulant activity in human plasma, to have good plasma exposure upon oral administration to mammals, and to possess particularly advantageous pharmacological and toxicological profiles of activity.

R^1 preferably represents trifluoromethyl, COOH, $CONH_2$, phenyl, pyridyl, N-(1-4C)alkylimidazol-4-yl or a cyclopropyl, cyclohexyl, oxetanyl, tetrahydropyranyl, azetidinyl or piperidinyl group, which azetidinyl or piperidinyl group may bear an N-(1-4C)alkyl substituent.

More preferably R^1 represents trifluoromethyl, COOH, $CONH_2$, phenyl, pyrid-2-yl, pyrid-3-yl, pyrid-4-yl, N-methylimidazol-4-yl, cyclopropyl, cyclohexyl, tetrahydropyran-4-yl or an N-methylpiperidin-4-yl group.

In the groups represented by R^2, X^2 preferably represents a hydrogen atom or a halogen atom.

More preferably X^2 represents a hydrogen atom or a fluorine atom;

X^3 represents a hydrogen atom, a fluorine atom, a chlorine atom or a methyl group;

X^4 represents a chlorine atom;

X^5 represents a chlorine atom or a methoxy group; and

X^6 represents a chlorine atom.

Particularly preferred values for R^2 are 4-chlorophenyl, 4-methoxyphenyl, 3-fluoro-4-methoxyphenyl, indol-6-yl, 3-methylindol-6-yl, 3-chloroindol-6-yl, 5-fluoroindol-2-yl, 5-chloroindol-2-yl or 6-chlorobenzo[b]thiophen-2-yl.

Especial mention may be made of compounds of formula (I) in which R^2 is 4-methoxyphenyl, indol-6-yl or 5-chloroindol-2-yl.

One particular value for X^1 is CH. Another is N.

A pharmaceutically acceptable metabolically labile ester of a compound of formula (I) is an ester formed between a carboxyl group (present in compounds of formula (I) when R^1 is COOH) and a pharmaceutically acceptable alcohol, which ester is hydrolyzed *in vivo* to afford the carboxylic acid and the alcohol. Examples of such esters include (1-6C) alkyl esters, such as methyl and ethyl esters.

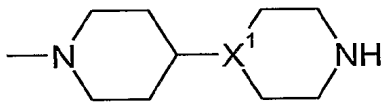
As used herein, unless otherwise indicated, the term halogen atom includes fluorine, chlorine and bromine.

It will be appreciated that the compounds of formula (I) contain a center of asymmetry that has the (D) configuration. The (D) configuration refers to the configuration of the amino acids from which the compounds may be prepared. The compounds may therefore exist and be isolated in a mixture with the corresponding (L) isomer, such as a racemic mixture, or separately. Preferably the compounds are isolated substantially free of the (L) isomer.

It will also be appreciated that the compounds of formula (I) or their pharmaceutically acceptable salts may be isolated in the form of a solvate, and accordingly that any such solvate is included within the scope of the present invention.

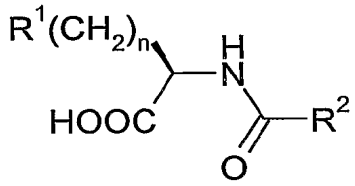
The compounds of formula (I) and their pharmaceutically acceptable salts may be prepared by a process, which comprises

(a) reacting a compound of formula (II)



(II)

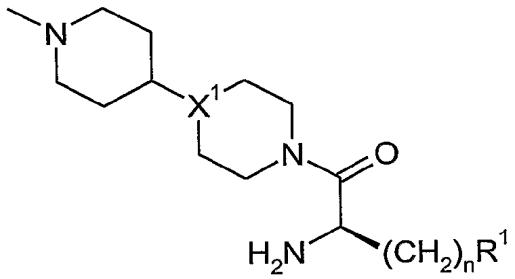
or a salt thereof, with a compound of formula (III)



(III)

or a reactive derivative thereof; or

(b) reacting a compound of formula (IV)



(IV)

or a salt thereof, with a compound of formula (V)

(V)

or a reactive derivative thereof;

followed, if a pharmaceutically acceptable metabolically labile ester or a pharmaceutically acceptable salt is desired, by forming a pharmaceutically acceptable metabolically labile ester or salt.

The reaction between a compound of formula (II) with a compound of formula (III) may conveniently be performed employing reagents and reaction conditions conventionally used for the formation of an amide bond. The reaction is conveniently carried out in the presence of a benzotriazole-based reagent such as 1-hydroxybenzotriazole or 1-hydroxy-7-azabenzotriazole and a dehydrating agent such as dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, in an inert organic solvent such as dimethylformamide and/or methylene chloride. The reaction is conveniently conducted at a temperature of from 0 to 50 °C, preferably at ambient temperature. If a salt of a compound of formula (II) is used, the reaction is conveniently performed in the additional presence of a base such as triethylamine. Other suitable reagents and solvents are known in the art, for example an acid halide, such as the chloride in the presence of a base, such as triethylamine.

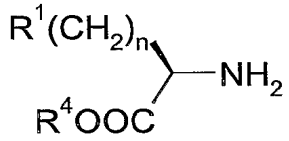
The reaction between a compound of formula (IV) with a compound of formula (V) may conveniently be performed employing reagents and reaction conditions conventionally used for the formation of an amide bond. The reaction is conveniently carried out in the presence of a benzotriazole-based reagent such as 1-hydroxybenzotriazole or 1-hydroxy-7-azabenzotriazole and a dehydrating agent such as dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, in an inert organic solvent such as dimethylformamide and/or methylene chloride. The reaction is conveniently conducted at a temperature of from 0 to 50 °C, preferably at ambient temperature. If a salt of a compound of formula (IV) is used, the reaction is conveniently performed in the additional presence of a base such as triethylamine. Other suitable reagents and solvents are known in the art, for example an acid halide, such as

p-anisoyl chloride in the presence of a base, such as triethylamine. Alternatively, the compound of formula (IV) may be reacted with a compound of formula (V) in the presence of diethylcyanophosphonate. This reaction is conveniently performed in an organic solvent such as dichloromethane in the presence of a base, such as triethylamine. The temperature is conveniently in the range of from -25 to 25°C.

The compound of formula (II) in which X^1 is CH is known, for example from WO 00/76971 at pages 163-164, and is named as 4-(1-methylpiperidin-4-yl)piperidine or 1-methyl-4,4'-bispiperidine.

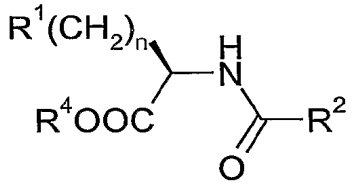
The compound of formula (II) in which X^1 is N is referred to herein as 1-(1-methylpiperidin-4-yl)piperazine.

The compounds of formula (III) may be prepared by reacting a compound of formula (VI)



(VI)

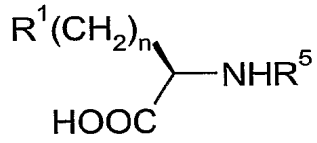
in which R^4 represents a carboxyl protecting group, for example a (1-6C)alkyl group, such as methyl or ethyl, with a compound of formula (V) to afford a compound of formula (VII)



(VII)

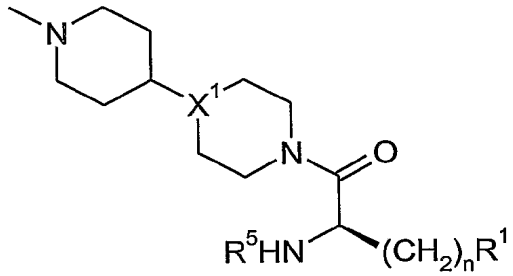
followed by removing the protecting group.

The compounds of formula (IV) may be prepared by reacting a compound of formula (II) with a compound of formula (VIII)



(VIII)

in which R^5 represents an amino protecting group, such as t-butoxycarbonyl (Boc) to afford a compound of formula (IX)



(IX)

followed by removing the protecting group.

The compounds of formulae (VI) and (VIII) are known or may be prepared using conventional methods for the preparation of amino acids protected on the carboxy or amino group. Particular preparations are also described in the Examples.

The compounds of formula (V) are well known.

The protection of amino and carboxylic acid groups is described in McOmie, Protecting Groups in Organic Chemistry, Plenum Press, NY, 1973, and Greene and Wuts, Protecting Groups in Organic Synthesis, 2nd. Ed., John Wiley & Sons, NY, 1991. Examples of carboxy protecting groups include C_1-C_6 alkyl groups such as methyl, ethyl, *t*-butyl and *t*-amyl; aryl(C_1-C_4)alkyl groups such as benzyl, 4-nitrobenzyl, 4-methoxybenzyl, 3,4-dimethoxybenzyl, 2,4-dimethoxybenzyl, 2,4,6-trimethoxybenzyl, 2,4,6-trimethylbenzyl, benzhydryl and trityl; silyl groups such as trimethylsilyl and *t*-butyldimethylsilyl; and allyl groups such as allyl and 1-(trimethylsilylmethyl)prop-1-en-3-yl.

Examples of amine protecting groups include acyl groups, such as groups of formula RCO in which R represents C_{1-6} alkoxy, phenyl C_{1-6} alkoxy, or a C_{3-10} cycloalkoxy, wherein a phenyl group may be optionally substituted, for example by one or two of halogen, C_1-C_4 alkyl and C_1-C_4 alkoxy.

Preferred amino protecting groups include benzyloxycarbonyl (CBz) and t-butoxycarbonyl (Boc).

Certain of the intermediates described herein, for example the compounds of formulae (III) and (IV), are believed to be novel and accordingly are provided as further aspects of the invention.

The compounds of the invention may be administered by any convenient route, e.g. into the gastrointestinal tract (e.g. rectally or orally), the nose, lungs, musculature or vasculature or transdermally. The compounds may be administered in any convenient administrative form, e.g. tablets, powders, capsules, solutions, dispersions, suspensions, syrups, sprays, suppositories, gels, emulsions, patches etc. Such compositions may contain components conventional in pharmaceutical preparations, e.g. diluents, carriers, pH modifiers, sweeteners, bulking agents, and further active agents. If parenteral administration is desired, the compositions will be sterile and in a solution or suspension form suitable for injection or infusion. Such compositions form a further aspect of the invention.

Viewed from this aspect the invention provides a pharmaceutical composition, which comprises the compound of formula (I) or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable diluent or carrier.

According to another aspect, the present invention provides the compound of formula (I) or a pharmaceutically acceptable salt thereof, for use in therapy.

According to another aspect, the present invention provides the use of the compound of formula (I) or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for the treatment of a thrombotic disorder.

According to another aspect, the present invention provides a method of treating a thrombotic disorder in a subject requiring treatment, which comprises administering an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof.

The subject may be a human or a non-human animal, such as a non-human mammal, for example a cat, dog, horse, cow or sheep.

The thrombotic disorder may be, for example, venous thrombosis, pulmonary embolism, arterial thrombosis, myocardial ischaemia, myocardial infarction or cerebral thrombosis. A particular indication is, for example, prophylaxis of post-operative venous thrombosis following high risk orthopedic surgery (such as hip or knee replacement), primary treatment of venous thrombosis, secondary prevention of ischemic cardiovascular complications following myocardial infarction (in combination with e.g. low dose aspirin), or prevention of embolic stroke in non-valvular atrial fibrillation. The compounds may also be used in accordance with the method of the invention in the treatment of acute vessel closure associated with thrombolytic therapy and restenosis, for example after transluminal coronary angioplasty or bypass grafting of the coronary or peripheral arteries, and in the maintenance of vascular access patency in long term hemodialysis patients.

The dosage of the compound of formula (I) will depend upon the nature and severity of the condition being treated, the administration route and the size and species of the

subject. In general, quantities in the range of from 0.01 to 100μM/kg bodyweight will be administered.

As used herein, the term "treatment" includes prophylactic use. The term "effective amount" refers to the amount of the compound of formula (I) that is effective to reduce or inhibit the development of the symptoms of the thrombotic disorder being treated.

The compound according to the invention may be administered alone or in combination with an anticoagulant having a different mode of action or with a thrombolytic agent.

The following Examples illustrate the invention.

API-MS (atmospheric pressure chemical ionization mass spectra) were obtained on a PESciex API 150EX with a heated nebulizer and nitrogen as the reagent gas in positive ion mode.

CI-MS (Chemical ionization mass spectra) were obtained on a Shimadzu 5000 direct insertion mass spectrometer in chemical ionization mode utilizing methane as the reagent gas.

TLC performed on AnalTech No. 02521 silica gel plates.

The following abbreviations are used throughout: Abbreviations used follow IUPAC-IUB nomenclature. Additional abbreviations are Boc, tertiary-butyloxycarbonyl; CMA, chloroform: methanol: concentrated ammonium hydroxide (80:18:2); DEPC, diethyl cyanophosphonate. DCC, dicyclohexylcarbodiimide; DIEA, *N,N*-diisopropylethylamine; DMSO, dimethyl sulfoxide (perdeuterated if for NMR); DMF, dimethylformamide; EDCI, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; ES-MS, electrospray mass spectrum; LCMS, liquid chromatography mass spectrum; EtOAc,

ethyl acetate; Et_2O, diethyl ether; HOAt, 1-hydroxy-7-aza-benzotriazole; HOBt, 1-hydroxybenzotriazole; HPLC, high pressure liquid chromatography; MeOH, methanol; SCX, strong cation exchange; TEA, triethylamine; TFA, trifluoroacetic acid; and THF, tetrahydrofuran. Reagents were obtained from a variety of commercial sources.

Method 1: A solution or suspension of an amine or amine hydrochloride salt (1 eq, approximately 0.2 M) in THF, dichloromethane, or DMF (or a mixture of any of these solvents) is treated with a carboxylic acid (approximately 1 eq), either HOBt or HOAt (approximately 1 eq), either TEA or DIEA (0-3 eq), and either EDCI or DCC (approximately 1 eq). After stirring overnight at room temperature, the solvents are removed and the residue is diluted with ethyl acetate or dichloromethane and washed with saturated aqueous sodium bicarbonate and brine. The organic solution is then dried with $MgSO_4$, filtered and concentrated in vacuo. If necessary, the product is purified by chromatography over silica gel, eluting with a gradient of 0% through 2 to 12% 2 N ammonia/methanol in dichloromethane or chloroform. The product-containing fractions are then combined and concentrated in vacuo.

Method 2: To a stirring solution of an amine or amine hydrochloride salt (1 eq), triethylamine (1-3 eq), and a carboxylic acid (about 1.2 eq) in dichloromethane (0.2-0.5 M) at 0 °C, is slowly added diethyl cyanophosphonate (about 1.2 eq). After stirring overnight, the solvents are removed in vacuo; and the residue is partitioned between water and an organic solvent such as ethyl acetate or dichloromethane and washed with saturated aqueous $NaHCO_3$, followed by brine. The organic phase is then dried with $MgSO_4$ or Na_2SO_4, filtered and concentrated in vacuo. If

necessary, the product is purified by chromatography over silica gel, eluting with a gradient of 0-10% 2 N ammonia/methanol in either dichloromethane or chloroform. The product-containing fractions are then combined and concentrated in vacuo.

Method 3: The amine or amine hydrochloride salt (1 eq) and triethylamine (1-3 eq) are dissolved in dichloromethane (0.2-0.5 M) and an acid chloride (about 1.2 eq) is added. After stirring for about 3 h, the volatiles are removed in vacuo; and the residue is dissolved in methanol (possibly with an organic cosolvent such as dichloromethane) and loaded onto a strong cation exchange (SCX) column. The column is washed with methanol, and then the desired product is eluted from the column with a solution of ammonia or triethylamine in methanol (possibly with an organic cosolvent such as dichloromethane). The product containing fractions are then combined and concentrated in vacuo. If necessary, the product is purified further by chromatography over silica gel, eluting with a gradient of 0-10% 2 N ammonia/methanol in either dichloromethane or chloroform. The product-containing fractions are then combined and concentrated in vacuo.

Method 5: A solution or suspension of an amine or amine hydrochloride salt (1 eq, approximately 0.2 M) in THF, dichloromethane, or DMF (or a mixture of any of these solvents) is treated with a carboxylic acid (approximately 1 eq), and either TEA or DIEA (0-3 eq) and mixed several minutes. Either HOBt or HOAt (approximately 1 eq) and either EDCI or DCC (approximately 1 eq) are separately stirred together in a solvent; and the resulting mixture is added to the other solution, or vice versa. After stirring overnight at room temperature, either the solvents are

removed and the residue is diluted with ethyl acetate or dichloromethane, or the reaction solution is partitioned between the reaction solvent and saturated aqueous sodium bicarbonate, separated, and the organics washed with saturated aqueous sodium bicarbonate and saturated brine. The organic solution is then dried with $MgSO_4$, filtered and concentrated in vacuo. If necessary, the product is purified by chromatography over silica gel, eluting with a gradient of 0% through 2 to 12% 2 N ammonia/methanol in dichloromethane or chloroform. The product-containing fractions are then combined and concentrated in vacuo.

General Deprotection Methods

Method 1: A solution of the t-butylcarbamate (1 eq) in CH_2Cl_2 (0.2 M) is treated with anisole (5 eq) and TFA (20% by volume). After stirring 1 to 3 h at ambient temperature, the reaction mixture is concentrated *in vacuo*. The crude residue is purified by strong cation exchange chromatography (SCX). The SCX column is washed with a 5% solution of acetic acid in methanol and the TFA salt is dissolved in methanol (possibly with a cosolvent such as dichloromethane) and loaded onto the SCX column. The column is then washed with methanol (possibly with a cosolvent such as dichloromethane), and then the free base is eluted from the column with a 2 N solution of ammonia or triethylamine in methanol (possibly with a cosolvent such as dichloro-methane). The product containing fractions are then combined and concentrated in vacuo to give the product in the free base form.

Method 2: HCl gas is bubbled into a solution of the t-butylcarbamate in anhydrous MeOH (0.1 M) for approximately 10 to 30 min, then the reaction mixture is either

concentrated *in vacuo* or filtered immediately and washed with ether to give the HCl salt of the title amine.

General HCl Salt Formation Methods

Method 1: The free base is dissolved in 0.2 N aqueous HCl (1-2 eq of HCl). The resulting solution is freeze-dried to give the amine hydrochloride salt.

Method 2: A solution of the free base in a small amount of CH_2Cl_2 is treated with 1.0-2.2 equivalents of 1 M HCl in ether. After stirring 30 min, the reaction mixture is filtered, and the resulting solid is rinsed with ether and dried to give the amine hydrochloride salt.

General Analytical HPLC Methods

Method 1: Vydac C18 (4.6 x 250 mm) or Symmetry (4.6 x 150 mm), elute with a linear gradient of 90/10 through 50/50 (0.1% TFA in water / 0.1% TFA in acetonitrile) over 45 min, 1 mL/min, λ=214 nm.

Method 2: Vydac C18 (4.6 x 250 mm), elute with a linear gradient of 90/10 through 50/50 (0.1% TFA in water / 0.1% TFA in acetonitrile) over 20 or 40 min, 1 mL/min, λ=all.

HPLC Analysis (Method A): Waters Symmetry, C18 (4.6 x 250 mm) column. The elution system consisted of linear gradient from 95:5 (0.1% TFA in H_2O)/(0.1% TFA in CH_3CN) to 5:95 (0.1% TFA in H_2O)/ (0.1% TFA in CH_3CN) over 20 min, followed by 5:95 (0.1% TFA in H_2O)/(0.2% TFA in CH_3CN) isocratic over 15 min. The flow rate was 1 mL/min. UV Detection was performed at 254 nm unless otherwise noted.

Preparation of Compounds of formula (VIII)

Preparation (VIII)-1.
***N*-Boc-β-(1-Methylpiperidin-4-yl)-D-alanine.**

A. *N*-Boc-D-β-(1-methylpyridin-4-ium)alanine iodide A mixture of *N*-Boc-β-(4-pyridyl)-D-alanine (4.0 g, 15.02 mmol) and iodomethane (3.19 g, 22.53 mmol) in acetone (50 mL) was heated at reflux for 16 h. The suspension was then concentrated under reduced pressure to give *N*-Boc-β-(1-methylpyridin-4-ium)-D-alanine iodide as a yellow foam (6.13 g, quantitative)
^{1}H NMR (CD_3OD).
APCI-MS, m/e = 283 [$C_{14}H_{21}N_2O_4$+1].

B. *N*-Boc-β-(1-Methylpiperidin-4-yl)-D-alanine

A mixture of *N*-Boc-β-(1-methylpyridin-4-ium)-D-alanine iodide (6.1 g, 14.94 mmol) and platinum(IV) oxide (0.10 g, 0.44 mmol) in methanol (50 mL) was placed under a hydrogen atmosphere (2.04 bar,30 psi) for 16 h on a Parr hydrogenation apparatus. The mixture was filtered over diatomaceous earth and poured over 50 g of SCX resin (activated with 5% acetic acid/methanol). The resin was washed with methanol (100 mL) and flushed with saturated ammonia in methanol solution (100 mL). The basic fraction was concentrated under reduced pressure to give the subtitled compound as a white foam (4.19 g, 98%).
^{1}H NMR (CD_3OD).
APCI-MS, m/e = 287 [$C_{14}H_{26}N_2O_4$+1].

Preparation of Compounds of formula (IX)

Preparation (IX)-1.

1-(N-Boc-β-Phenyl-D-alanyl)-4-(1-methylpiperidin-4-yl)-piperazine.

Prepared from Boc-β-phenyl-D-alanine and 1-(1-methyl-piperidin-4-yl)piperazine using methods substantially equivalent to General Coupling Method 5.

^{1}H NMR.

ES-MS, m/z 430.3 $(M+1)^+$.

Analysis for $C_{25}H_{39}N_3O_3 \cdot 1.3H_2O$.

Calcd: C 66.28; H 9.26; N 9.27.

Found: C 66.07; H 8.79; N 9.28.

Preparation (IX)-2.

1-[N-Boc-(γ-Benzyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)-piperidine.

Prepared from N-Boc-D-glutamatic acid γ-benzyl ester and 4-(1-methylpiperidin-4-yl)piperidine dihydrobromide using methods substantially equivalent to General Coupling Method 1.

^{1}H NMR.

ES-MS, m/z 502.4$(M+1)^+$.

Analysis For $C_{28}H_{43}N_3O_5 \cdot 1.0H_2O$:

Calcd: C 64.71; H 8.73; N 8.09.

Found: C 65.07; H 8.43; N 8.47.

Preparation (IX)-3.

1-[N-Boc-(β-Benzyl)-D-aspartyl]-4-(1-methylpiperidin-4-yl)-piperidine.

Prepared from N-Boc-D-aspartic acid β-benzyl ester and 4-(1-methylpiperidin-4-yl)piperidine dihydrobromide using

methods substantially equivalent to General Coupling Method 1.

^{1}H NMR.

ES-MS, m/z 488.4$(M+1)^+$.

Analysis For $C_{21}H_{39}N_3O_4 \cdot 1.0H_2O$:

Calcd: C, 66.50; H 8.47; N 8.62.

Found: C, 65.85; H 8.19; N 8.71.

Preparation (IX)-4.

1-[*N*-Boc-β-(3-Pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine.

Method B-1: To a suspension of *N*-Boc-β-(3-pyridinyl)-D-alanine (1.0 g, 3.76 mmol) and 4-(1-methylpiperidin-4-yl)-piperidine dihydrobromide (1.18 g, 3.42 mmol) in anhydrous dichloromethane (30 mL) under nitrogen atmosphere was added DEPC (0.66 g, 4.10 mmol) at −15 °C. The mixture was stirred for 20 min; then *N,N*-diisopropylethylamine was added. The mixture was stirred for 16 h at room temperature. The organic layer was washed with 20 mL portions of saturated aqueous sodium bicarbonate solution, water and brine. The organic layer was subsequently dried over sodium sulfate, filtered, and concentrated under reduced pressure to give a crude yellow oil. The oil was purified by flash column chromatography over silica gel, eluting with dichloromethane/CMA (10:1 to 3:1), to give the titled compound as a colorless gum (0.56 g, 38%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 431 (M+1).

Preparation (IX)-5.

1-[*N*-Boc-β-(4-Pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine.

Using methods substantially equivalent to those described in Method B-1, the titled compound was prepared from *N*-Boc-β-(4-pyridinyl)-D-alanine and 4-(1-methyl-piperidin-4-yl)piperidine dihydrobromide (44%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 431 (M+1).

Preparation (IX)-6.

1-[*N*-Boc-β-(2-Pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine.

Using methods substantially equivalent to those described in Method B-1, the titled compound was prepared from *N*-Boc-β-(2-pyridinyl)-D-alanine and 4-(1-methyl-piperidin-4-yl)piperidine dihydrobromide (33%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 431 (M+1).

Preparation (IX)-7.

1-(*N*-Boc-1-Methyl-D-histidinyl)-4-(1-methylpiperidin-4-yl)-piperidine.

Using methods substantially equivalent to those described in Method B-2, the subtitled compound was prepared from *N*-Boc-1-methyl-D-histidine and 4-(1-methylpiperidin-4-yl)piperidine (72%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e 434 ($C_{23}H_{39}N_5O_3$+1).

Preparation (IX)-8.

1-[*N*-Boc-β-Cyclohexyl-D-alanyl)-4-(1-methylpiperidin-4-yl)-piperidine.

Method B-3: To a mixture of *N*-Boc-β-cyclohexyl-D-alanine (1.0 g, 3.7 mmol), 4-(1-methylpiperidin-4-yl)piperidine

dihydrobromide (1.5 g, 4.47 mmol), HOBt (0.5 g, 3.7 mmol) and EDCI (0.85 g, 4.43 mmol) in DMF (5.6 mL) was added diisopropylethylamine (2.6 mL, 16 mmol); and the mixture stirred overnight at room temperature. The solvent was removed under vacuum. The residue was suspended in water and extracted with dichloromethane. The organic layer was washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum. The residue was purified by chromatography on silica gel with dichloromethane/CMA to provide the titled compound (600 mg, 34%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 436 (M+1).

Preparation (IX)-9.

1-[N-Boc-β-(4-tetrahydropyranyl)alanyl]-4-(1-methyl-piperidin-4-yl)piperidine.

Using methods substantially equivalent to that described in Method B-3, the titled compound was prepared from N-Boc-β-(4-tetrahydropyranyl)alanine and 4-(1-methyl-piperidin-4-yl)piperidine (22%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 438 (M+1).

Preparation (IX)-10.

1-(N-Boc-β-Cyclohexyl-D-alanyl)-4-(1-methylpiperidin-4-yl)-piperazine.

Prepared from Boc-β-cyclohexyl-D-alanine and 1-(1-methylpiperidin-4-yl)piperazine using methods substantially equivalent to General Coupling Method 1.

^{1}H NMR.

ES-MS, m/z 437.5 $(M+1)^+$.

Analysis For $C_{24}H_{44}N_4O_3 \cdot 1.0H_2O$.

Calcd: C 63.40; H 10.20; N 12.32.

Found: C 63.84; H 9.78; N 12.69.

Preparation (IX)-11.

1-[N-Boc-β-(4-Tetrahydropyranyl)alanyl]-4-(1-methyl-piperidin-4-yl)piperazine.

Prepared from N-Boc-β-(4-tetrahydropyranyl)alanine and 1-(1-methylpiperidin-4-yl)piperazine using methods substantially equivalent to General Coupling Method 1.

^1H NMR.

ES-MS, 439.4 m/z $(M+1)^+$.

Analysis for $C_{23}H_{42}N_4O_4 \cdot 1.0H_2O$.

Calcd: C 60.50; H 9.71; N 12.27.

Found: C 61.08; H 9.26; N 12.86.

Preparation (IX)-12.

1-[*N*-Boc-β-(4-Pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine.

Method B-2: To a solution of N-Boc-β-(4-pyridyl)-D-alanine (3.0 g, 11.3 mmol) and 1-(1-methylpiperidin-4-yl)piperazine (2.07 g, 11.3 mmol) in anhydrous *N,N*-dimethylformamide (20 mL) under nitrogen atmosphere at 0 °C was added HOBt (1.52 g, 11.3 mmol) followed by *N,N*-diisopropylethylamine (2.91 g, 22.5 mmol). 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (2.37 g, 12.4 mmol) was added, and the mixture stirred for 16 h at room temperature. The mixture was diluted with water (100 mL) and washed four times with 50 mL portions of chloroform/2-propanol (3:1). The organic layer was washed with 50-mL portions of water and brine and dried over anhydrous sodium sulfate. The organic layer was filtered and concentrated under reduced pressure to give a crude oil. The oil was purified by flash chromatography, eluting with dichloromethane/CMA (50:1 to 3:1), to give the titled compound as a white foam (3.88 g, 80%).

^1H NMR ($CDCl_3$).

APCI-MS, m/e = 432 (M+1).

Preparation (IX)-13.

1-[*N*-Boc-β-(2-Pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method B-2, the titled compound was prepared from *N*-Boc-β-(2-pyridinyl)-D-alanine and 1-(1-methylpiperidin-4-yl)piperazine (72%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 432 (M+1).

Preparation (IX)-14.

1-(*N*-Boc-D-Glutamyl)-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method B-2, the titled compound was prepared from *N*-Boc-D-glutamine and 1-(1-methylpiperidin-4-yl)-piperazine (67%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 412 (M+1).

Preparation (IX)-15.

1-[*N*-Boc-β-(1-Methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method B-2, the titled compound was prepared from *N*-Boc-β-(1-methylpiperidin-4-yl)-D-alanine and 1-(1-methylpiperidin-4-yl)piperazine (57%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 452 (M+1).

Preparation (IX)-16.

1-(N-Boc-D-Asparaginyl)-4-(1-methylpiperidin-4-yl)-piperazine.

Using methods substantially equivalent to those described in Method B-2, the titled compound was prepared from *N*-Boc-D-asparagine and 1-(1-methylpiperidin-4-yl)-piperazine (66%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 398 (M+1).

Preparation (IX)-17.

1-[N-Boc-β-(Trifluoromethyl)-D/L-alanyl-4-(1-methyl-piperidin-4-yl)piperazine.

Boc-D,L-trifluoromethylalanine (1.3g, 5.05mmole), (1-methylpiperidin-4-yl)piperazine (0.77g, 4.21mmole), HOAt (0.74g, 5.47mmole), EDCI (1.05g, 5.47mmole) and triethylamine (1.4ml, 10mmole) were dissolved in DMF (30ml) and stirred overnight at room temperature. All volatiles were removed under high vacuum and the residue partitioned between sat. aqueous sodium bicarbonate and 4:1 chloroform/isopropyl alcohol. The organic solution was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The oil obtained was purified by flash chromatography (SiO2, DCM:MeOH:10%:ammonia solution - 80:10:10) to give 1-(Boc-D,L-trifluoromethylalaninyl)-4-(1-methylpiperidin-4-yl)piperazine (0.83g).

^{1}H NMR

LCMS 423 $(M+1)^+$

Preparation (IV)-1.

1-(β-Phenyl-D-alanyl)-4-(1-methylpiperidin-4-yl)piperidine.

Prepared from 1-(N-Boc-β-phenyl-D-alanyl)-4-(1-methyl-piperidin-4-yl)piperidine using methods substantially equivalent to General Deprotection Method 1.

^{1}H NMR (DMSO-d_6) 10.8 (bs, 1 H), 8.42 (m, 3 H), 7.29 (m, 5 H), 4.60 (bs, 1 H), 4.38 (bd, J = 12.8 Hz, 1 H), 4.09 (bs, 1 H), 3.63 (m, 1 H), 3.33 (d, J = 11.7 Hz, 2 H), 3.2 (m 1 H), 2.96 - 2.74 (m, 3 H), 2.64 (d, J = 4.4 Hz, 3 H), 2.37 (m, 1 H), 2.20 (m, 0.5 H), 1.8 - 0.8 (m, 8.5 H), 0.63 (m, 0.5 H), -0.29 (m, 0.5 H).

MS (ES+) 329.2 m/z

Preparation (IV)-2.

1-[(γ-Methyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)-piperidine Dihydrochloride.

Prepared from 1-[N-Boc-(γ-benzyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)piperidine using methods substantially equivalent to those described in General Deprotection Method 2, which resulted in removal of the Boc group and transesterification of the ester.

^{1}H NMR.

ES-MS, m/z 326.5 $(M+1)^+$.

Preparation (IV)-3.

1-[(β-Benzyl)-D-aspartyl]-4-(1-methylpiperidin-4-yl)-piperidine Hydrochloride.

Prepared from 1-[N-Boc-(β-benzyl)-D-aspartyl]-4-(1-methylpiperidin-4-yl)piperidine using methods substantially equivalent to those described in General Deprotection Method 2.

^{1}H NMR.

ES-MS, m/z 388.5 $(M+1)^+$.

Analysis For $C_{22}H_{33}N_3O_3 \cdot 2.0HCl \cdot 1.7H_2O$.

Calcd: C 53.81; H 7.88; N 8.56 Cl 14.40.

Found: C 53.40; H 8.15; N 8.61 Cl 14.16.

Preparation (IV)-4.
1-[β-(3-Pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)-piperidine Trihydrochloride.

Method C-1: To a solution of 1-[N-Boc-β-(3-pyridinyl)-D-alanyl)-4-(1-methylpiperidin-4-yl)piperidine (0.740 g, 1.71 mmol) and anisole (4.08 g, 37.8 mmol) in methanol (10 mL) was added concentrated hydrochloric acid (2.0 mL) at 0 °C. The mixture was stirred for 5 h at room temperature. The mixture was concentrated under reduced pressure to give the titled compound as a white foam (0.739 g, quantitative).
^{1}H NMR (CD_3OD).
APCI-MS, m/e = 331 ($C_{19}H_{30}N_4O$+1).

Preparation (IV)-5.
1-[β-(4-Pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)-piperidine Trihydrochloride.

Using methods substantially equivalent to those described in Method C-1, the titled compound was prepared from 1-[N-Boc-β-(4-pyridinyl)-D-alanyl]-4-(1-methyl-piperidin-4-yl)piperidine (quantitative).
^{1}H NMR (CD_3OD).
APCI-MS, m/e = 331 ($C_{19}H_{30}N_4O$+1).

Preparation (IV)-6.
1-[β-(2-Pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)-piperidine Trihydrochloride.

Using methods substantially equivalent to those described in Method C-1, the subtitled compound was prepared from 1-[N-Boc-β-(2-pyridinyl)alanyl]-4-(1-methylpiperidin-4-yl)piperidine (quantitative).

^{1}H NMR (CD_3OD).
APCI-MS, m/e = 331 ($C_{19}H_{30}N_4O$+1).

Preparation (IV)-7.

1-(1-Methyl-D-histidinyl)-4-(1-methylpiperidin-4-yl)-piperidine Trihydrochloride.

Using methods substantially equivalent to those described in Method C-1, the subtitled compound was prepared from 1-(*N*-Boc-1-methyl-D-histidinyl)-4-(1-methylpiperidin-4-yl)piperidine (72%).
^{1}H NMR (CD_3OD).
APCI-MS, m/e 334 ($C_{18}H_{31}N_5O$+1).

Preparation (IV)-8.

1-(β-Cyclohexyl-D-alanyl)-4-(1-methylpiperidin-4-yl)-piperidine Dihydrochloride.

Method C-2: A mixture of 1-(*N*-Boc-β-cyclohexyl-D-alanyl)-4-(1-methylpiperidin-4-yl)piperidine (3.4 g, 9 mmol), methanol (50 mL), and anisole (15 mL) was cooled to 0 °C. Concentrated HCl (20 mL) was added. The mixture was stirred 2 h at room temperature. The mixture was concentrated under vacuum and the residue triturated in diethyl ether. The solids were collected by vacuum filtration to provide the subtitled compound as a white solid (0.55 g, 98%).
^{1}H NMR (CD_3OD).
APCI-MS, m/e = 336 (M+1).

Preparation (IV)-9.

1-[β-(4-Tetrahydropyranyl)alanyl]-4-(1-methylpiperidin-4-yl)piperidine Dihydrochloride.

Using methods substantially equivalent to that described in Method C-2, the titled compound was prepared

from 1-(*N*-Boc-β-(4-tetrahydropyranyl)alanyl]-4-(1-methyl-piperidin-4-yl)piperidine (98%).

^{1}H NMR (CD_3OD).

APCI-MS, m/e = 338 (M+1).

Preparation (IV)-10

1-(β-Cyclohexyl-D-alanyl)-4-(1-methylpiperidin-4-yl)-piperazine Hydrochloride.

Prepared from 1-(N-Boc-β-cyclohexyl-D-alanyl)-4-(1-methylpiperidin-4-yl)piperazine using methods substantially equivalent to those described in General Deprotection Method 2.

^{1}H NMR.

ES-MS, m/z 337.3 $(M+1)^+$.

Analysis For $C_{19}H_{36}N_4O \cdot 3.0HCl \cdot 2.0H_2O$:

Calcd: C 47.35; H 8.99; N 11.63.

Found: C 47.73; H 8.28; N 11.79.

Preparation (IV)-11.

1-[β-(4-Tetrahydropyranyl)alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Prepared from 1-[N-Boc-β-(4-tetrahydropyranyl)alanyl]-4-(1-methylpiperidin-4-yl)piperazine using methods substantially equivalent to those described in General Deprotection Method 2.

^{1}H NMR.

ES-MS, m/z 339.4 $(M+1)^+$.

Analysis For $C_{18}H_{34}N4O_2 \cdot 3.0HCl \cdot 4.0H_2O$:

Calcd: C 41.58; H 8.72; N 10.78 Cl 20.46.

Found: C 41.40; H 7.58; N 10.81 Cl 20.54.

Preparation (IV)-12.

1-[β-(4-Pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)-piperazine Tetrahydrochloride.

Using methods substantially equivalent to those described in Method C-1, the titled compound was prepared from 1-[N-Boc-β-(4-pyridinyl)-D-alanyl]-4-(1-methyl-piperidin-4-yl)piperazine (quantitative).

^{1}H NMR (CD_3OD).

APCI-MS, m/e = 333 ($C_{18}H_{29}N_5O$+1).

Preparation (IV)-13.

1-[β-(2-Pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)-piperazine Tetrahydrochloride.

Using methods substantially equivalent to those described in Method C-1, the titled compound was prepared from 1-[N-Boc-β-(2-pyridinyl)-D-alanyl)-4-(1-methyl-piperidin-4-yl)piperazine (quantitative).

^{1}H NMR (CD_3OD).

APCI-MS, m/e = 333 ($C_{18}H_{29}N_5O$+1).

Preparation (IV)-14.

1-(D-Glutamyl)-4-(1-methylpiperidin-4-yl)piperazine Trihydrochloride.

Using methods substantially equivalent to those described in Method C-2, the subtitled compound was prepared from 1-(N-Boc-D-glutamyl)-4-(1-methylpiperidin-4-yl)-piperazine (quantitative).

^{1}H NMR (CD_3OD).

APCI-MS, m/e = 315 ($C_{15}H_{29}N_5O_2$+1).

Preparation (IV)-15.

1-[β-(1-Methylpiperidin-4-yl)-D-alanyl]-4-(1-methyl-piperidin-4-yl)piperazine Tetrahydrochloride.

Using methods substantially equivalent to those described in Method C-2, the titled compound was prepared from 1-[*N*-Boc-β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine (quantitative).

^{1}H NMR (CD_3OD).

APCI-MS, m/e = 354 ($C_{19}H_{37}N_5O$+1).

Preparation (IV)-16.

1-(D-Asparaginyl)-4-(1-methylpiperidin-4-yl)piperazine Trihydrochloride.

Using methods substantially equivalent to those described in Method C-2, the titled compound was prepared from 1-(N-Boc-D-asparaginyl)-4-(1-methylpiperidin-4-yl)-piperazine (quantitative).

^{1}H NMR (CD_3OD).

APCI-MS, m/e = 353 ($C_{14}H_{27}N_5O_2$+1).

Preparation (IV)-17.

1-β-(Trifluoromethyl)-D/L-alanyl-4-(1-methylpiperidin-4-yl)-piperazine.

1-(Boc-D,L-trifluoromethylalaninyl)-4-(1-methylpiperidin-4-yl)piperazine (0.83g) was dissolved in ethyl acetate (30ml) and HCl gas bubbled in for 10min. Methanol (20ml) was added to help dissolve the precipitate formed. When the reaction was complete (LCMS) the solution was evaporated to dryness to give the trihydrochloride salt (840mg). This was converted to the free base by absorption onto an SCX ion exchange column and elution with a solution of ammonia in methanol/dichloromethane to give 1-(D,L-trifluoromethylalaninyl)-4-(1-methylpiperidin-4-yl)piperazine 660mg.

^{1}H NMR.

Example 1.

1-[N-(Indole-6-carbonyl)-β-phenyl-D-alanyl]-4-(1-methyl-piperidin-4-yl)piperidine Hydrochloride.

Prepared from 1-(β-phenyl-D-alanyl)-4-(1-methyl-piperidin-4-yl)piperidine hydrochloride and indole-6-carboxylic acid using methods substantially equivalent to General Coupling Method 5. The HCl salt is prepared following Salt Formation Method 2.

^{1}H NMR.

ES-MS, m/z 476.3 $(M+1)^+$; 471.1 $(M-1)^-$.

Analysis For $C_{29}H_{36}N_4O_2 \cdot 1.0HCl \cdot 2.0H_2O$.

Calcd: C 63.90; H 7.58; N 10.28; Cl 6.50.

Found: C 63.93; H 7.26; N 10.00; Cl 6.35.

Analytical HPLC (Method 1): >96%, t_r = 25.4 min.

Example 2.

1-[N-(4-Methoxybenzoyl)-β-phenyl-D-alanyl]-4-(1-methyl-piperidin-4-yl)piperidine Hydrochloride.

Prepared from 1-(β-phenyl-D-alanyl)-4-(1-methyl-piperidin-4-yl)piperidine hydrochloride and 4-methoxybenzoic acid using methods substantially equivalent to General Coupling Method 5. The HCl salt is prepared following Salt Formation Method 2.

^{1}H NMR.

ES-MS, m/z 464.1 $(M+1)^+$.

Analysis For $C_{28}H_{37}N_3O_3 \cdot 1.5HCl$.

Calcd: C 64.88; H 7.49; N 8.11; Cl 10.26.

Found: C 64.64; H 7.47; N 7.94; Cl 9.98.

Analytical HPLC (Method 1): >99%, t_r = 24.1 min.

Example 3.

1-[N-(3-Chloroindole-6-carbonyl)-β-phenyl-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine Hydrochloride.

Prepared from 1-(β-phenyl-D-alanyl)-4-(1-methyl-piperidin-4-yl)piperidine hydrochloride and 3-chloroindole-6-carboxylic acid using methods substantially equivalent to General Coupling Method 1. The HCl salt is prepared following Salt Formation Method 2.

^1H NMR.

ES-MS, m/z 507.3 $(M+1)^+$; 505.3 $(M-1)^-$.

Analysis For $C_{29}H_{35}ClN_4O_2 \cdot 1.1HCl \cdot 1.0H_2O$.

Calcd: C 61.63; H 6.80; N 9.91; Cl 13.17.

Found: C 61.60; H 6.58; N 9.92; Cl 13.50.

Analytical HPLC (Method 1): >99%, t_r = 30.2 min.

Example 4.

1-[N-(5-Chloroindole-2-carbonyl)-β-phenyl-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine Hydrochloride.

Prepared from 1-β-phenyl-D-alanyl-4-(1-methylpiperidin-4-yl)piperidine hydrochloride and 5-chloroindole-2-carboxylic acid using methods substantially equivalent to General Coupling Method 1. The HCl salt is prepared following Salt Formation Method 2.

^1H NMR.

ES-MS, m/z 507.3 $(M+1)^+$; 505.3 $(M-1)^-$.

Analysis For $C_{29}H_{35}ClN_4O_2 \cdot 1.1HCl \cdot 1.0H_2O$.

Calcd: C 61.63; H 6.80; N 9.91; Cl 13.17.

Found: C 61.15; H 6.64; N 9.63; Cl 13.04.

Analytical HPLC (Method 1): >98%, t_r = 34.3 min.

Example 5.

1-[N-(3-Methylindole-6-carbonyl)-β-phenyl-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine Hydrochloride.

Prepared from 1-(β-phenyl-D-alanyl)-4-(1-methyl-piperidin-4-yl)piperidine hydrochloride and 3-methylindole-6-carboxylic acid using methods substantially equivalent to

General Coupling Method 1. The HCl salt is prepared following Salt Formation Method 2.

^{1}H NMR.

ES-MS, m/z 487.4 $(M+1)^+$; 485.4 $(M-1)^-$.

Analysis For $C_{30}H_{38}N_4O_2 \cdot 1.15HCl \cdot 1.1H_2O$.

Calcd: C 65.70; H 7.60; N 10.21; Cl 7.44.

Found: C 65.42; H 7.32; N 10.19; Cl 7.33.

Analytical HPLC (Method 1): >96%, t_r = 29.2 min.

Example 6.

1-[N-(4-Chlorobenzoyl)-β-phenyl-D-alanyl]-4-(1-methyl-piperidin-4-yl)piperidine Hydrochloride.

Prepared from 1-(β-phenyl-D-alanyl)-4-(1-methyl-piperidin-4-yl)piperidine hydrochloride and 4-chlorobenzoyl chloride using methods substantially equivalent to General Coupling Method 3. The HCl salt is prepared following Salt Formation Method 2.

^{1}H NMR.

ES-MS, m/z 468.4 $(M+1)^+$; 466.4 $(M-1)^-$.

Analysis For $C_{27}H_{34}ClN_3O_2 \cdot 1.0HCl \cdot 0.5H_2O$.

Calcd: C 64.09; H 7.27; N 8.30; Cl 14.01.

Found: C 63.65; H 7.07; N 8.19; Cl 13.93.

Analytical HPLC (Method 1): >97%, t_r = 29.3 min.

Example 7.

1-[N-(Indole-6-carbonyl)-(γ-methyl)-D-glutamyl]-4-(1-methyl-piperidin-4-yl)piperidine Hydrochloride.

Prepared from 1-[(γ-methyl)-D-glutamyl]-4-(1-methyl-piperidin-4-yl)piperidine dihydrochloride and indole-6-carboxylic acid using methods substantially equivalent to General Coupling Method 1. The HCl salt is prepared following General Salt Formation Method 2.

^{1}H NMR.

ES-MS, m/z 469.5$(M+1)^+$; 467.5 $(M-1)^-$.

Analysis For $C_{26}H_{36}N_4O_4 \cdot 1HCl \cdot 1.0H_2O$.

Calcd: C 59.70; H 7.52; N 10.71.

Found: C 59.73; H 7.49; N 10.45.

Analytical HPLC (Method 1): >96%, t_r = 16.6 min.

Example 8.

1-[N-(5-Chloroindole-2-carbonyl)-(γ-methyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)piperidine Hydrochloride.

Prepared from 1-[(γ-methyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)piperidine dihydrochloride and 5-chloroindole-2-carboxylic acid using methods substantially equivalent to General Coupling Method 1. The HCl salt is prepared following Salt Formation Method 2.

^1H NMR.

ES-MS, m/z 503.5 $(M+1)^+$; 501.5 $(M-1)^-$.

Analytical HPLC (Method 1): >96%, t_r = 25.8 min.

Example 9.

1-[N-(Indole-6-carbonyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)piperidine Hydrochloride.

Prepared from 1-[(γ-methyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)piperidine dihydrochloride and indole-6-carboxylic acid using methods substantially equivalent to General Coupling Method 1. Ester deprotection with 2 eq LiOH and final purification and HCl salt formation via prep HPLC.

^1H NMR.

ES-MS, m/z 455.4 $(M+1)^+$; 453.5 $(M-1)^-$.

Analysis For $C_{25}H_{34}N_4O_4 \cdot 0.8HCl \cdot 3.5H_2O$.

Calcd: C 53.74; H 7.40; N 10.03; Cl 5.08.

Found: C 53.20; H 6.90; N 9.90; Cl 4.73.

Analytical HPLC (Method 1): >99%, t_r = 13.0 min.

Example 10.

1-[N-(5-Chloroindole-2-carbonyl)-D-glutamyl]-4-(1-methyl-piperidin-4-yl)piperidine Hydrochloride.

Prepared from 1-[(γ-methyl)-D-glutamyl]-4-(1-methyl-piperidin-4-yl)piperidine dihydrochloride hydrochloride and 5-chloroindole-2-carboxylic acid using methods substantially equivalent to General Coupling Method 1. Ester deprotection with 2 eq LiOH and final purification and HCl salt formation via prep HPLC.

^{1}H NMR.

ES-MS, m/z 489.4 $(M+1)^+$; 487.4 $(M-1)^-$.

Analysis For $C_{25}H_{33}ClN_4O_4 \cdot 0.3HCl \cdot 1.7H_2O$.

Calcd: C 56.12; H 6.86; N 10.47; Cl 8.61.

Found: C 55.67; H 7.05; N 10.51; Cl 8.65.

Analytical HPLC (Method 1): >99%, t_r = 23.2 min.

Example 11.

1-[N-(Indole-6-carbonyl)-D-aspartyl]-4-(1-methylpiperidin-4-yl)piperidine hydrochloride.

Prepared from 1-[(β-benzyl)-D-aspartyl]-4-(1-methyl-piperidin-4-yl)piperidine Hydrochloride and indole-6-carboxylic acid using methods substantially equivalent to General Coupling Method 1. Ester deprotection with 2 eq LiOH and final purification and HCl salt formation via prep HPLC.

^{1}H NMR.

ES-MS, m/z 441.4$(M+1)^+$; 439.4 $(M-1)^-$.

Analytical HPLC (Method 1): >99%, t_r = 12.3 min.

Example 12a.

1-[N-(Indole-6-carbonyl)-β-(3-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine.

Method D-1: To a solution of 1-[β-(3-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine trihydrochloride (0.850 g, 1.93 mmol) and indole-6-carboxylic acid (0.311 g, 1.93 mmol) in *N,N*-dimethylformamide (15 mL) under nitrogen atmosphere was added 1-hydroxybenzotriazole (0.261 g, 1.93 mmol) and *N,N*-diisopropylethylamine (0.749 g, 5.79 mmol) at 0 °C. The mixture was stirred for 10 min at 0 °C then EDCI (0.408 g, 2.13 mmol) was added. The mixture was stirred for 16 h at room temperature. The mixture was diluted with water (100 mL) and washed four times with 50 mL portions of chloroform/2-propanol (3:1). The organic layer was washed with 50 mL portions of water and brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure to give a crude oil. The oil was purified by flash chromatography, eluting with dichloromethane/CMA (50:1 to 3:1), to give the titled compound as a clear oil (0.250 g, 27%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 474 (M+1).

Example 12b.

1-[N-(Indole-6-carbonyl)-β-(3-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine Hydrochloride.

Salt Formation Method 3: To a solution of 1-[N-(indole-6-carbonyl)-β-(3-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine (0.370 g, 0.782 mmol) in acetonitrile (5 mL) at 0 °C was slowly added hydrochloric acid (1 M solution in diethyl ether, 0.782 mL, 0.782 mmol). The mixture was stirred for 10 minutes at 0 °C and was concentrated under reduced pressure to give the titled compound as an off-white solid (0.378 g, 95%).

$[\alpha]^{25}_D$ +7.0° (*c* = 0.5, Methanol).

Melting Point = 177-182 °C with decomposition.

^{1}H NMR (CD_3OD).
APCI-MS, m/e = 474 [$C_{28}H_{35}N_5O_2$+1].
Analysis for $C_{28}H_{35}N_5O_2 \cdot 1.1HCl \cdot 2.9H_2O$:
Calcd: C, 59.42; H, 7.46; N, 12.12; Cl, 6.89.
Found: C, 59.65; H, 7.46; N, 12.12; Cl, 7.01.
HPLC Analysis (Method A) : 98.4% t_r = 9.4 min.
TLC Analysis: R_f = 0.27 (1:1 Dichloromethane/CMA).

Example 13a.

1-[N-(Indole-6-carbonyl)-β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine.

Using methods substantially equivalent to those described in Method D-1, the titled compound was prepared from 1-[β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine trihydrochloride and indole-6-carboxylic acid (23%).
^{1}H NMR ($CDCl_3$).
APCI-MS, m/e = 474 (M+1).

Example 13b.

1-[N-(Indole-6-carbonyl)-β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(indole-6-carbonyl)-β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine (96%).
$[\alpha]^{25}_D$ +16.0° (*c* 0.5, Methanol).
Melting Point = 183-186 °C with decomposition.
^{1}H NMR (CD_3OD).
APCI-MS, m/e = 474 [$C_{28}H_{35}N_5O_2$+1].
Analysis for $C_{28}H_{35}N_5O_2 \cdot 1.1HCl \cdot 2.5H_2O$:
Calcd: C, 60.19; H, 7.41; N, 12.53; Cl, 6.98.
Found: C, 60.54; H, 7.32; N, 12.59; Cl, 7.03.

HPLC Analysis (Method A) : >99% t_r = 8.7 min.
TLC Analysis: R_f = 0.29 (1:1 Dichloromethane/CMA).

Example 14a.

1-[N-(Indole-6-carbonyl)-β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine.

Using methods substantially equivalent to those described in Method D-1, the subtitled compound was prepared from 1-[β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine trihydrochloride and indole-6-carboxylic acid (32%).
^{1}H NMR ($CDCl_3$).
APCI-MS, m/e = 474 (M+1).

Example 14b.

1-[N-(Indole-6-carbonyl)-β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(indole-6-carbonyl)-β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine (95%).
$[\alpha]^{25}_D$ +13.8° (*c* 0.5, Methanol).
Melting Point = 175-179 °C with decomposition.
^{1}H NMR (CD_3OD).
APCI-MS, m/e = 474 [$C_{28}H_{35}N_5O_2$+1].
Analysis for $C_{28}H_{35}N_5O_2 \cdot 1.1HCl \cdot 2.8H_2O$:

Calcd:	C, 59.61;	H, 7.45;	N, 12.41;	Cl, 6.91.
Found:	C, 59.47;	H, 7.43;	N, 12.28;	Cl, 7.07.

HPLC Analysis (Method A) : >99% t_r = 8.9 min.
TLC Analysis: R_f = 0.30 (1:1 Dichloromethane/CMA).

Example 15a.

1-[N-(Indole-6-carbonyl)-1-methyl-D-histidinyl]-4-(1-methyl-piperidin-4-yl)piperidine.

Using methods substantially equivalent to those described in Method D-1, the subtitled compound was prepared from 1-(1-methyl-D-histidinyl)-4-(1-methylpiperidin-4-yl)-piperidine trihydrochloride and indole-6-carboxylic acid (23%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e 477 ($C_{27}H_{36}N_6O_2$+1).

Example 15b.

1-[N-(Indole-6-carbonyl)-1-methyl-D-histidinyl]-4-(1-methyl-piperidin-4-yl)piperidine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(indole-6-carbonyl)-1-methyl-D-histidinyl]-4-(1-methylpiperidin-4-yl)piperidine (94%).

$[\alpha]^{25}_D$ +46.7° (*c* 0.25, Methanol)

Melting Point = 179-185 °C with decomposition.

^{1}H NMR (CD_3OD).

APCI-MS, m/e 477 ($C_{27}H_{36}N_6O_2$+1).

TLC R_f = 0.67 (3:7 CH_2Cl_2:CMA)

Analysis for $C_{27}H_{36}N_6O_2 \cdot 1.6HCl \cdot 4.1H_2O$:

Calcd: C, 53.27; H, 7.58; N, 13.80; Cl, 9.32.

Found: C, 53.47; H, 7.55; N, 13.58; Cl, 9.51.

HPLC Analysis (Method A): >99% t_r = 9.6 min.

Example 16a.

1-[N-(Indole-6-carbonyl)-β-cyclohexyl-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine.

Using methods substantially equivalent to that described in Method D-1, the titled compound was prepared

from 1-(β-cyclohexyl-D-alanyl)-4-(1-methylpiperidin-4-yl)-piperidine dihydrochloride and indole-6-carboxylic acid (65%).

^{1}H NMR ($CDCl_3$).

TLC R_f = 0.37 (5:2 CH_2Cl_2:CMA)

Example 16b.

1-[N-(Indole-6-carbonyl)-β-cyclohexyl-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(indole-6-carbonyl)-β-cyclohexyl-D-alanyl]-4-(1-methylpiperidin-4-yl)piperidine (96%).

$[\alpha]^{25}_D$ -32.7° (*c* 0.20, Methanol)

Melting Point = 162-172 °C with decomposition.

^{1}H NMR (CD_3OD).

APCI-MS, m/e = 479 ($C_{29}H_{42}N_4O_2$+1).

TLC R_f = 0.37 (5:2 CH_2Cl_2:CMA)

Analysis for $C_{29}H_{42}N_4O_2 \cdot HCl \cdot 1.7H_2O$:

Calcd: C, 63.82; H, 8.57; N, 10.27; Cl, 6.50.

Found: C, 63.66; H, 8.63; N, 10.26; Cl, 6.75.

HPLC Analysis (Method A): >99% t_r = 15.8 min.

Example 17a.

1-[N-(Indole-6-carbonyl)-β-(4-tetrahydropyranyl)alanyl]-4-(1-methylpiperidin-4-yl)piperidine.

Using methods substantially equivalent to that described in Method D-1, the subtitled compound was prepared from 1-[β-(4-tetrahydropyranyl)alanyl]-4-(1-methylpiperidin-4-yl)-piperidine dihydrochloride and indole-6-carboxylic acid (69%).

^{1}H NMR ($CDCl_3$).

TLC R_f = 0.19 (5:2 CH_2Cl_2:CMA)

Example 17b.

1-[N-(Indole-6-carbonyl)-β-(4-tetrahydropyranyl)alanyl]-4-(1-methylpiperidin-4-yl)piperidine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(indole-6-carbonyl)-β-(4-tetrahydropyranyl)alanyl]-4-(1-methylpiperidin-4-yl)piperidine (96%).
Melting Point = 162-178 °C with decomposition.
^{1}H NMR (CD_3OD).
APCI-MS, m/e = 481 ($C_{28}H_{40}N_4O_3$+1).
TLC R_f = 0.19 (5:2 CH_2Cl_2:CMA)
Analysis for $C_{28}H_{40}N_4O_3 \cdot 1.1HCl \cdot 2.5H_2O$:
Calcd: C, 59.44; H, 8.21; N, 9.90; Cl, 6.89.
Found: C, 59.60; H, 8.38; N, 9.84; Cl, 6.74.
HPLC Analysis (Method A): >99% t_r = 11.7 min.

Example 18.

1-[N-(6-Chlorobenzo[b]thiophene-2-carbonyl)-β-cyclohexyl-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Prepared from 1-(β-cyclohexyl-D-alanyl)-4-(1-methylpiperidin-4-yl)piperazine hydrochloride and 6-chlorobenzo[b]thiophene-2-carboxylic acid using methods substantially equivalent to General Coupling Method 1. The HCl salt is prepared following Salt Formation Method 1.
^{1}H NMR.
ES-MS, m/z 531.4 $(M+1)^+$; 529.4 $(M-1)^-$.
Analysis For $C_{28}H_{39}ClN_4O_2S \cdot 1.1HCl \cdot 4.0H_2O$.
Calcd: C 52.27; H 7.54; N 8.71.
Found: C 51.83; H 6.58; N 8.53.
Analytical HPLC (Method 1): >99%, t_r = 32.6 min.

Example 19.

1-[N-(5-Chloroindole-2-carbonyl)-β-cyclohexyl-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Prepared from 1-(β-cyclohexyl-D-alanyl)-4-(1-methylpiperidin-4-yl)piperazine hydrochloride and 5-chloroindole-2-carboxylic acid using methods substantially equivalent to General Coupling Method 1. The HCl salt is prepared following Salt Formation Method 1.

^{1}H NMR.

ES-MS, m/z 514.2 $(M+1)^+$; 512.3 $(M-1)^-$.

Analysis For $C_{28}H_{40}ClN_5O_2 \cdot 1.0HCl \cdot 3.5H_2O$.

Calcd: C 54.81; H 7.89; N 11.41.

Found: C 54.92; H 6.93; N 11.20.

Analytical HPLC (Method 1): >99%, t_r = 31.6 min.

Example 20.

1-[N-(Indole-6-carbonyl)-β-cyclohexyl-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Prepared from 1-(β-cyclohexyl-D-alanyl)-4-(1-methylpiperidin-4-yl)piperazine hydrochloride and indole-6-carboxylic acid using methods substantially equivalent to General Coupling Method 1. The HCl salt is prepared following General Salt Formation Method 1.

^{1}H NMR.

ES-MS, m/z 480.3 $(M+1)^+$; 478.3 $(M-1)^-$.

Analysis For $C_{28}H_{41}N_5O_2 \cdot 1.1HCl \cdot 3.5H_2O$.

Calcd: C 57.70; H 8.49; N 12.02.

Found: C 57.39; H 7.89; N 11.78.

Analytical HPLC (Method 1): >99%, t_r = 25.1 min.

Example 21.

1-[N-(6-Chlorobenzo[b]thiophene-2-carbonyl)-β-(4-tetrahydropyranyl)alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Prepared from 1-[β-(4-tetrahydropyranyl)alanyl]-4-(1-methylpiperidin-4-yl)piperazine hydrochloride and 6-chlorobenzo[b]thiophene-2-carboxylic acid using methods substantially equivalent to General Coupling Method 1. The HCl salt is prepared following Salt Formation Method 1.

^{1}H NMR.

ES-MS, m/z 533.2 $(M+1)^+$; 531.3 $(M-1)^-$.

Analysis For $C_{27}H_{37}ClN_4O_3S \cdot 1.0HCl \cdot 2.0H_2O$.

Calcd: C 53.55; H 6.99; N 9.25.

Found: C 53.16; H 6.46; N 9.34.

Analytical HPLC (Method 1): >99%, t_r = 24.1 min.

Example 22.

1-[N-(5-Chloroindole-2-carbonyl)-β-(4-tetrahydropyranyl)-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Prepared from 1-[β-(4-tetrahydropyranyl)alanyl]-4-(1-methylpiperidin-4-yl)piperazine hydrochloride and 5-chloroindole-2-carboxylic acid using methods substantially equivalent to General Coupling Method 1. The HCl salt is prepared following Salt Formation Method 1.

^{1}H NMR.

ES-MS, m/z 516.2 $(M+1)^+$; 514.3 $(M-1)^-$.

Analysis For $C_{25}H_{36}N_4O_2 \cdot 1.1HCl \cdot 2.0H_2O$.

Calcd: C 54.09; H 7.28; N 11.68.

Found: C 54.34; H 6.83; N 11.69.

Analytical HPLC (Method 1): >97%, t_r = 23.2 min.

Example 23.

1-[N-(Indole-6-carbonyl)-β-(4-tetrahydropyranyl)alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Prepared from 1-[β-(4-tetrahydropyranyl)alanyl]-4-(1-methylpiperidin-4-yl)piperazine hydrochloride and indole-2-carboxylic acid using methods substantially equivalent to General Coupling Method 1. The HCl salt is prepared following General Salt Formation Method 1.

^{1}H NMR.

ES-MS, m/z 482.3 $(M+1)^+$; 480.3 $(M-1)^-$.

Analysis For $C_{27}H_{39}N_5O_3 \cdot 1.1HCl \cdot 3.5H_2O$.

Calcd: C 55.45; H 8.12; N 11.98.

Found: C 55.20; H 7.06; N 11.94.

Analytical HPLC (Method 1): >99%, t_r = 15.5 min.

Example 24a.

1-[N-(Indole-6-carbonyl)-β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the titled compound was prepared from 1-[β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine tetrahydrochloride and indole-6-carboxylic acid (50%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 475 (M+1).

Example 24b.

1-[N-(Indole-6-carbonyl)-β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(indole-6-carbonyl)-β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine (96%).

$[\alpha]^{25}_D$ +25.0° (*c* 0.4, Methanol).
Melting Point = 228-235 °C with decomposition.
^1H NMR (CD_3OD).
APCI-MS, m/e = 475 [$C_{27}H_{34}N_6O_2$+1].
Analysis for $C_{27}H_{34}N_6O_2 \cdot 1.5HCl \cdot 2.2H_2O \cdot 0.1CH_2Cl_2$:
Calcd: C, 56.37; H, 7.00; N, 14.55; Cl, 10.44.
Found: C, 56.71; H, 7.01; N, 14.15; Cl, 10.25.
HPLC Analysis (Method A) : >99% t_r = 8.1 min.
TLC Analysis: R_f = 0.36 (1:1 Dichloromethane/CMA).

Example 25a.
1-[N-(6-Chlorobenzo[b]thiophene-2-carbonyl)-β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the titled compound was prepared from 1-[β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine tetrahydrochloride and 6-chloro-benzo[b]thiophene-2-carboxylic acid (49%).
^1H NMR ($CDCl_3$).
APCI-MS, m/e = 527 (M+1).

Example 25b.
1-[N-(6-Chlorobenzo[b]thiophene-2-carbonyl)-β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(6-chlorobenzo[b]thiophene-2-carbonyl)-β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine (94%).
$[\alpha]^{25}_D$ +7.4° (*c* 0.05, Methanol).
Melting Point = 213-217 °C with decomposition.
^1H NMR (CD_3OD).

APCI-MS, m/e = 527 [$C_{27}H_{32}ClN_5O_2S$+1].

Analysis for $C_{27}H_{32}ClN_5O_2S \cdot 1.9HCl \cdot 1.4H_2O$:

Calcd: C, 52.26; H, 5.96; N, 11.29; Cl, 16.57.

Found: C, 52.41; H, 6.07; N, 11.08; Cl, 16.77.

HPLC Analysis (Method A) : >99% t_r = 9.4 min.

TLC Analysis: R_f = 0.46 (1:1 Dichloromethane/CMA).

Example 26a.

1-[N-(5-Chloroindole-2-carbonyl)-β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the titled compound was prepared from 1-[β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine tetrahydrochloride and 5-chloroindole-2-carboxylic acid (47%).

^1H NMR ($CDCl_3$).

APCI-MS, m/e = 510 (M+1).

Example 26b.

1-[N-(5-Chloroindole-2-carbonyl)-β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(5-chloroindole-2-carbonyl)-β-(4-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine (94%).

$[\alpha]^{25}_D$ +30.6° (*c* 0.17, Methanol).

Melting Point = 238-242 °C with decomposition.

^1H NMR (CD_3OD).

APCI-MS, m/e = 509 [$C_{27}H_{33}ClN_6O_2$+1].

Analysis for $C_{27}H_{33}ClN_6O_2 \cdot 2.2HCl \cdot 1.8H_2O$:

Calcd: C, 52.16; H, 6.29; N, 13.52; Cl, 18.25.

Found: C, 52.26; H, 6.12; N, 13.28; Cl, 18.23.

HPLC Analysis (Method A) : >99% t_r = 10.9 min.
TLC Analysis: R_f = 0.33 (1:1 Dichloromethane/CMA).

Example 27a.

1-[N-(6-Chlorobenzo[b]thiophene-2-carbonyl)-β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the titled compound was prepared from 1-[β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine tetrahydrochloride and 6-chlorobenzo[b]thiophene-2-carboxylic acid (53%).
^1H NMR ($CDCl_3$).
APCI-MS, m/e = 427 (M+1).

Example 27b.

1-[N-(6-Chlorobenzo[b]thiophene-2-carbonyl)-β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(6-chlorobenzo[b]thiophene-2-carbonyl)-β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine (96%).
$[\alpha]^{25}_D$ +7.4° (*c* 0.05, Methanol).
Melting Point = 219-223 °C with decomposition.
^1H NMR (CD_3OD).
APCI-MS, m/e = 526 [$C_{27}H_{32}ClN_5O_2S$+1].
Analysis for $C_{27}H_{32}ClN_5O_2S \cdot 1.4HCl \cdot 1.75H_2O$:
Calcd: C, 53.28; H, 6.11; N, 11.51; Cl, 13.98.
Found: C, 53.50; H, 6.03; N, 11.30; Cl, 13.86.
HPLC Analysis (Method A) : 98.9% t_r = 9.9 min.
TLC Analysis: R_f = 0.46 (1:1 Dichloromethane/CMA).

Example 28a.

1-[N-(Indole-6-carbonyl)-β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the titled compound was prepared from 1-[β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine tetrahydrochloride and indole-6-carboxylic acid (62%).

^1H NMR ($CDCl_3$).

APCI-MS, m/e = 475 (M+1).

Example 28b.

1-[N-(Indole-6-carbonyl)-β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(indole-6-carbonyl)-β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine (94%).

$[\alpha]^{25}_D$ +2.3° (*c* 0.05, Methanol).

Melting Point = 232-235 °C with decomposition.

^1H NMR (CD_3OD).

APCI-MS, m/e = 475 [$C_{27}H_{34}N_6O_2$+1].

Analysis for $C_{27}H_{34}N_6O_2 \cdot 1.75HCl \cdot 3.9H_2O$:

Calcd: C, 53.28; H, 7.21; N, 13.81; Cl, 10.19.

Found: C, 53.33; H, 7.18; N, 13.70; Cl, 10.26.

HPLC Analysis (Method A) : 98.5% t_r = 6.9 min.

TLC Analysis: R_f = 0.32 (1:1 Dichloromethane/CMA).

Example 29a.

1-[N-(5-Chloroindole-2-carbonyl)-β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the subtitled compound was prepared

from 1-[β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine tetrahydrochloride and 5-chloroindole-2-carboxylic acid (54%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 510 (M+1).

Example 29b.

1-[N-(5-Chloroindole-2-carbonyl)-β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(5-chloroindole-2-carbonyl)-β-(2-pyridinyl)-D-alanyl]-4-(1-methylpiperidin-4-yl)-piperazine (94%).

Melting Point = 210-214 °C with decomposition.

^{1}H NMR (CD_3OD).

APCI-MS, m/e = 509 [$C_{27}H_{33}ClN_6O_2$+1].

Analysis for $C_{27}H_{33}ClN_6O_2 \cdot 2.25HCl \cdot 1.2H_2O$:

Calcd: C, 52.93; H, 6.19; N, 13.72; Cl, 18.81.

Found: C, 52.06; H, 6.12; N, 13.51; Cl, 18.67.

HPLC Analysis (Method A) : >99% t_r = 10.9 min.

TLC Analysis: R_f = 0.38 (1:1 Dichloromethane/CMA).

Example 30a.

1-[N-(Indole-6-carbonyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the titled compound was prepared from 1-(D-glutamyl)-4-(1-methylpiperidin-4-yl)piperazine trihydrochloride and indole-6-carboxylic acid (46%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 455 (M+1).

Example 30b.

1-[N-(Indole-6-carbonyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(indole-6-carbonyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)piperazine (96%).

Melting Point = 215-218 °C with decomposition.

^1H NMR (CD_3OD).

APCI-MS, m/e = 455 [$C_{24}H_{34}N_6O_3$+1].

Analysis for $C_{24}H_{34}N_6O_3 \cdot 2.0HCl \cdot 3.1H_2O$:

Calcd: C, 49.42; H, 7.21; N, 14.41; Cl, 12.16.

Found: C, 49.67; H, 7.43; N, 14.13; Cl, 11.89.

HPLC Analysis (Method A) : >99% t_r = 14.7 min.

TLC Analysis: R_f = 0.32 (CMA).

Example 31a.

1-[N-(6-Chlorobenzo[b]thiophene-2-carbonyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the subtitled compound was prepared from 1-(D-glutamyl)-4-(1-methylpiperidin-4-yl)piperazine trihydrochloride and 6-chlorobenzo[b]thiophene-2-carboxylic acid (41%).

^1H NMR ($CDCl_3$).

APCI-MS, m/e = 507 (M+1).

Example 31b.

1-[N-(6-Chlorobenzo[b]thiophene-2-carbonyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(6-chlorobenzo[b]thiophene-2-

carbonyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)piperazine (93%).

Melting Point = 185-190 °C with decomposition.

^{1}H NMR (CD_3OD).

APCI-MS, m/e = 506 [$C_{24}H_{32}ClN_5O_3S$+1].

Analysis for $C_{24}H_{32}ClN_5O_3S \cdot 1.4HCl \cdot 2.6H_2O$:

Calcd: C, 47.73; H, 6.44; N, 11.60; Cl, 14.09.

Found: C, 47.58; H, 6.37; N, 11.52; Cl, 14.07.

HPLC Analysis (Method A) : >99% t_r = 11.9 min.

TLC Analysis: R_f = 0.34 (CMA).

Example 32a.

1-[N-(5-Chloroindole-2-carbonyl)-D-glutamyl]-4-(1-methyl-piperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the titled compound was prepared from 1-(D-glutamyl)-4-(1-methylpiperidin-4-yl)piperazine trihydrochloride and 5-chloroindole-2-carboxylic acid (43%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 489 (M+1).

Example 32b.

1-[N-(5-Chloroindole-2-carbonyl)-D-glutamyl]-4-(1-methyl-piperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(5-chloroindole-2-carbonyl)-D-glutamyl]-4-(1-methylpiperidin-4-yl)piperazine (96%).

Melting Point = 222-225 °C with decomposition.

^{1}H NMR (CD_3OD).

APCI-MS, m/e = 489 [$C_{24}H_{33}ClN_6O_3$+1].

Analysis for $C_{24}H_{33}ClN_6O_3 \cdot 2.0HCl \cdot 2.3H_2O$:

Calcd: C, 47.78; H, 6.61; N, 13.93; Cl, 17.63.

Found: C, 47.99; H, 6.86; N, 13.57; Cl, 17.60.

HPLC Analysis (Method A) : 95.2% t_r = 11.4 min.
TLC Analysis: R_f = 0.23 (CMA).

Example 33a.

1-[N-(6-Chlorobenzo[b]thiophene-2-carbonyl)-β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)-piperazine.

Using methods substantially equivalent to those described in Method D-1, the titled compound was prepared from 1-[β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine tetrahydrochloride and 6-chlorobenzo[b]thiophene-2-carboxylic acid (52%).
^{1}H NMR ($CDCl_3$).
APCI-MS, m/e = 547 (M+1).

Example 33b.

1-[N-(6-Chlorobenzo[b]thiophene-2-carbonyl)-β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)-piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the subtitled compound was prepared from 1-[N-(6-chlorobenzo[b]thiophene-2-carbonyl)-β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine (96%).
Melting Point = 220-223 °C with decomposition.
^{1}H NMR (CD_3OD).
APCI-MS, m/e = 547 [$C_{28}H_{40}ClN_5O_2S$+1].
Analysis for $C_{28}H_{40}ClN_5O_2S \cdot 2.4HCl \cdot 2.0H_2O$:
Calcd: C, 50.22; H, 6.98; N, 10.46; Cl, 18.00.
Found: C, 49.96; H, 6.79; N, 10.34; Cl, 18.13.
HPLC Analysis (Method A) : 97.5% t_r = 11.2 min.
TLC Analysis: R_f = 0.34 (CMA).

Example 34a.

1-[N-(Indole-6-carbonyl)-β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the subtitled compound was prepared from 1-[β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine tetrahydrochloride and indole-6-carboxylic acid (59%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 495 (M+1).

Example 34b.

1-[N-(Indole-6-carbonyl)-β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(indole-6-carbonyl)-β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)-piperazine (98%).

Melting Point = 190-193 °C with decomposition.

^{1}H NMR (CD_3OD).

APCI-MS, m/e = 495 [$C_{28}H_{42}N_6O_2$+1].

Analysis for $C_{28}H_{42}N_6O_2 \cdot 1.7HCl \cdot 1.8H_2O$:

Calcd: C, 57.09; H, 8.09; N, 14.27; Cl, 10.23.

Found: C, 57.27; H, 8.41; N, 14.05; Cl, 10.20.

HPLC Analysis (Method A) : 98.7% t_r = 8.4 min.

TLC Analysis: R_f = 0.33 (CMA).

Example 35a.

1-[N-(5-Chloroindole-2-carbonyl)-β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the titled compound was prepared

from 1-[β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methyl-piperidin-4-yl)piperazine tetrahydrochloride and 5-chloro-indole-2-carboxylic acid (46%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 529 (M+1).

Example 35b.

1-[N-(5-Chloroindole-2-carbonyl)-β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(5-chloroindole-2-carbonyl)-β-(1-methylpiperidin-4-yl)-D-alanyl]-4-(1-methylpiperidin-4-yl)-piperazine (96%).

Melting Point = 205-210 °C with decomposition.

^{1}H NMR (CD_3OD).

APCI-MS, m/e = 529 [$C_{28}H_{41}ClN_6O_2$+1].

Analysis for $C_{28}H_{41}ClN_6O_2 \cdot 1.5HCl \cdot 1.7H_2O$:

Calcd: C, 54.24; H, 7.53; N, 13.68; Cl, 14.42.

Found: C, 54.48; H, 7.21; N, 13.54; Cl, 14.22.

HPLC Analysis (Method A) : 96.6% t_r = 10.9 min.

TLC Analysis: R_f = 0.35 (CMA).

Example 36a.

1-[N-(Indole-6-carbonyl)-D-asparaginyl]-4-(1-methyl-piperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the titled compound was prepared from 1-(D-asparaginyl)-4-(1-methylpiperidin-4-yl)piperazine trihydrochloride and indole-6-carboxylic acid (45%).

^{1}H NMR ($CDCl_3$).

APCI-MS, m/e = 451 (M+1).

Example 36b.

1-[N-(Indole-6-carbonyl)-D-asparaginyl]-4-(1-methyl-piperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the titled compound was prepared from 1-[N-(indole-6-carbonyl)-D-asparaginyl]-4-(1-methylpiperidin-4-yl)piperazine (95%).

Melting Point = 215-219 °C with decomposition.

^1H NMR (CD_3OD).

APCI-MS, m/e = 441 [$C_{23}H_{32}N_6O_3$+1].

Analysis for $C_{23}H_{32}N_6O_3 \cdot 1.5HCl \cdot 3.0H_2O$:

Calcd: C, 50.29; H, 7.25; N, 15.30; Cl, 9.68.

Found: C, 50.53; H, 7.14; N, 15.00; Cl, 9.68.

HPLC Analysis (Method A) : 96.2% t_r = 8.7 min.

TLC Analysis: R_f = 0.21 (CMA).

Example 37a.

1-[N-(6-Chlorobenzo[b]thiophene-2-carbonyl)-D-asparaginyl]-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the subtitled compound was prepared from 1-(D-asparaginyl)-4-(1-methylpiperidin-4-yl)piperazine trihydrochloride and 6-chlorobenzo[b]thiophene-2-carboxylic acid (40%).

^1H NMR ($CDCl_3$).

APCI-MS, m/e = 493 (M+1).

Example 37b.

1-[N-(6-Chlorobenzo[b]thiophene-2-carbonyl)-D-asparaginyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the subtitled compound was prepared from 1-[N-(6-chlorobenzo[b]thiophene-2-

carbonyl)-D-asparaginyl]-4-(1-methylpiperidin-4-yl)-piperazine (98%).
Melting Point = 219-223 °C with decomposition.
^{1}H NMR (CD_3OD).
APCI-MS, m/e = 493 [$C_{23}H_{30}ClN_5O_3S$+1].
Analysis for $C_{23}H_{30}ClN_5O_3S \cdot 1.4HCl \cdot 2.6H_2O$:
Calcd: C, 47.25; H, 6.05; N, 11.98; Cl, 16.37.
Found: C, 47.13; H, 5.86; N, 11.88; Cl, 16.29.
HPLC Analysis (Method A) : 95.9% t_r = 11.7 min.
TLC Analysis: R_f = 0.29 (CMA).

Example 38a.
1-[N-(5-Chloroindole-2-carbonyl)-D-asparaginyl])-4-(1-methylpiperidin-4-yl)piperazine.

Using methods substantially equivalent to those described in Method D-1, the subtitled compound was prepared from 1-[D-asparaginyl)-4-(1-methylpiperidin-4-yl)piperazine trihydrochloride and 5-chloroindole-2-carboxylic acid (46%).
^{1}H NMR ($CDCl_3$).
APCI-MS, m/e = 475 (M+1).

Example 38b.
1-[N-(5-Chloroindole-2-carbonyl)-D-asparaginyl]-4-(1-methyl-piperidin-4-yl)piperazine Hydrochloride.

Using methods substantially equivalent to those described in Salt Formation Method 3, the subtitled compound was prepared from 1-[N-(5-chloroindole-2-carbonyl)-D-asparaginyl]-4-(1-methylpiperidin-4-yl)piperazine (98%).
Melting Point = 235-240 °C with decomposition.
^{1}H NMR (CD_3OD).
APCI-MS, m/e = 475 [$C_{23}H_{31}ClN_6O_3$+1].
Analysis for $C_{23}H_{31}ClN_6O_3 \cdot HCl \cdot H_2O$:
Calcd: C, 47.25; H, 6.05; N, 11.98; Cl, 16.37.
Found: C, 47.13; H, 5.86; N, 11.88; Cl, 16.29.

HPLC Analysis (Method A) : >99% t_r = 11.8 min.
TLC Analysis: R_f = 0.25 (CMA).

Example 39.

1-[N-(Indole-6-carbonyl)-β-(trifluoromethyl)-D/L-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

1-(D,L-trifluoromethylalaninyl)-4-(1-methylpiperidin-4-yl)piperazine (330mg), indole-6-carboxylic acid (200mg), HOAt (180mg), EDCI (260mg) and triethylamine (0.5ml) were dissolved in DMF and stirred overnight. All volatiles were removed under high vacuum and the residue partitioned between sat. aqueous sodium bicarbonate and 4:1 chloroform/isopropyl alcohol. The organic solution was washed with brine and dried ($MgSO_4$) and concentrated. The product thus obtained was purified by reverse phase HPLC and converted to the free base by absorption onto an SCX ion exchange column and elution with a solution of ammonia in methanol to give 1-(indole-6-carbonyl-D,L-trifluoromethylalaninyl)-4-(1-methylpiperidin-4-yl)-piperazine (279mg).
^1H NMR
LCMS m/z 466 $(M+1)^+$
Analytical HPLC Luna C_{18} 3□m (4.6 x 30mm column), linear gradient 18% to 90% acetonitrile in water with 0.1% TFA over 5min: >95% t_r = 1.99min

Example 40.

1-[N-(5-Chloroindole-2-carbonyl)-β-(trifluoromethyl)-D/L-alanyl]-4-(1-methylpiperidin-4-yl)piperazine Hydrochloride.

Prepared from 1-(D,L-trifluoromethylalaninyl)-4-(1-methylpiperidiny-4-yl)piperazine and 5-chloroindole-2-carboxylic acid using methods substantially equivalent to that described above for 1-(indole-6-carbonyl-D,L-

trifluoromethylalaninyl)-4-(1-methylpiperidin-4-yl)-piperazine.

^{1}H NMR

LCMS m/z 500 $(M+1)^+$

Analytical HPLC Luna C_{18} 3□m (4.6 x 30mm column), linear gradient 18% to 90% acetonitrile in water with 0.1% TFA over 5min: >95% t_r = 2.51min

Enzyme Inhibition assays:

The ability of a test compound to inhibit factor Xa may be evaluated in one or more of the following Enzyme Inhibition assays, or in other standard assays known to those skilled in the art.

Enzyme Inhibition Assay

Human factor Xa and human thrombin are purchased from Enzyme Research Laboratories (South Bend, Indiana, USA). Other proteases are from other commercial sources. Chromogenic para-nitroanilide peptide protease substrates are purchased from Midwest Biotech (Fishers, Indiana, USA).

The binding affinities for human factor Xa are measured as apparent association constants (Kass) derived from protease inhibition kinetics as described previously.[a,b,c,d] The apparent Kass values are obtained using automated (BioMek-1000) dilutions of inhibitors (Kass determinations are performed in triplicate at each of four-eight inhibitor concentrations) into 96-well plates and chromogenic substrate hydrolysis rates determined at 405 nm using a Thermomax plate reader from Molecular Devices (San Francisco). For factor Xa inhibition, the assay protocol is: 50 μL buffer (0.06 M tris, 0.3 M NaCl, pH 7.4); 25 μL inhibitor test solution (in MeOH); 25 μL human factor Xa (32 nM in 0.03 M tris, 0.15 M NaCl, 1 mg/mL HSA); finally, 150 μL BzIleGluGlyArgpNA (0.3 mM in water) added within 2

min to start hydrolysis. Final [factor Xa] is 3.2 nM. [Free Xa] and [bound Xa] are determined from linear standard curves on the same plate by use of SoftmaxPro software for each inhibitor concentration and apparent Kass calculated for each inhibitor concentration which produced hydrolysis inhibition between 20% and 80% of the control (3.2 nM factor Xa): apparent Kass = $[E:I]/[E_f][I_f] = [E_b]/[E_f][I^o-I_b]$. The apparent Kass values so obtained are approximately the inverse of the Ki for the respective inhibitors [1/appKass = app Ki]. The variability of mean apparent Kass values determined at the single substrate concentration is +/- 15%. The assay system Km was measured as 0.347 +/- 0.031 mM [n=4]; and Vmax was 13.11 +/- 0.76 μM/min.

Kass values are determined with thrombin and other proteases using the same protocol with the following enzyme and substrate concentrations:
thrombin, 5.9 nM with 0.2 mM BzPheValArgpNA;
factor XIa, 1.2 nM with 0.4 mM pyroGluProArgpNA;
factor XIIa, 10 nM with 0.2 mM HDProPheArgpNA;
plasmin, 3.4 nM with 0.5 mM HDValLeuLyspNA;
nt-PA, 1.2 nM with 0.8 mM HDIleProArgpNA;
urokinase, 0.4 nM with 0.4 mM pyroGluGlyArgpNA;
aPC, 3 nM with 0.174 mM pyroGluProArgpNA;
plasma kallikrein, 1.9 nM with D-ProPheArgpNA; and
bovine trypsin, 1.4 nM with 0.18 mM BzPheValArgpNA.

Citations

(a) Sall DJ, JA Bastian, SL Briggs, JA Buben, NY Chirgadze, DK Clawson, ML Denny, DD Giera, DS Gifford-Moore, RW Harper, KL Hauser, VJ Klimkowski, TJ Kohn, H-S Lin, JR McCowan, AD Palkowitz, GF Smith, ME Richett, K Takeuchi, KJ Thrasher, JM Tinsley, BG Utterback, S-CB Yan, M Zhang. Dibasic Benzo[b]thiophenes Derivatives

as a Novel Class of Active Site Directed Thrombin Inhibitors. 1. Determination of the Serine Protease Selectivity, Structure-Activity Relationships and Binding Orientation. J Med Chem 40 3489-3493 (1997).

(b) Smith GF, TJ Craft, DS Gifford-Moore, WJ Coffman, KD Kurz, E Roberts, RT Shuman, GE Sandusky, ND Jones, N Chirgadze, and CV Jackson. A Family of Arginal Thrombin Inhibitors Related to Efegatran. Sem. Thrombos. Hemost. 22, 173-183 (1996).

(c) Smith GF, DS Gifford-Moore, TJ Craft, N Chirgadze, KJ Ruterbories, TD Lindstrom, JH Satterwhite. Efegatran: A New Cardiovascular Anticosagulant. In New Anticoagulants for the Cardiovascular Patient. Ed. R Pifarre. Hanley & Belfus, Inc., Philadelphia (1997) pp 265-300.

(d) Sall DJ, DL Bailey, JA Bastian, NY Chirgadze, AC Clemens-Smith, ML Denney, MJ Fisher, DD Geira, DS Gifford-Moore, RW Harper, LM Johnson, VJ Klimkowski, TJ Kohn, HS Lin, JR McCowan, AD Palkowitz, ME Richett, GF Smith, DW Snyder, K Takeuchi, JE Toth, M Zang. Diamino Benzo[b]thiophene Derivatives as a Novel Class of Active Site Directed Thrombin Inhibitors: 5. Potency, Efficacy and Pharmacokinetic Properties of Modified C-3 Side Chain Derivatives. J. Med. Chem., 43, 649-663 (2000).

The compounds of formula (I) exemplified herein have been found to exhibit a Kass of greater than 1 x 10^6 L/mole in the enzyme inhibition assay. For example, the compounds, or their pharmaceutically acceptable salts exemplified herein have been to exhibit Kass values of greater than 1 x 10^6 L/mole.

The ability of a test compound to elongate Partial Thromboplastin Time (Prothrombin Time) may be evaluated in the following test protocols.

Partial Thromboplastin Time (Prothrombin) Test Protocol

Venous blood is collected into 3.2% (0.109 M) trisodium citrate vacutainer tubes at 1 volume of anticoagulant to nine volumes of blood. The blood cells are separated by centrifugation at 700 g for ten minutes to yield plasma, which is frozen at 70 °C until required.

To perform the test, 100 µL of plasma are pipetted into in a glass test tube, 1 µL of test compound in DMSO is added, and allowed to warm to 37 ° over two minutes. 100 µL of warm (37 °) Manchester (tissue thromboplastin) reagent (Helena Biosciences, UK) is added, allowed to equilibrate for two minutes. 100 µL of warm (37 °) 25mM calcium chloride solution is added to initiate clotting. The test tube is tilted three times through a 90° angle every five seconds to mix the reagents and the time to clot formation recorded. Data from a series of observations and test compound concentrations are analysed by a SAS statistical analysis program and a CT2 (Concentration required to double clotting time) for each compound is generated.

Compounds of the invention have been found to significantly elongate the partial thromboplastin time (Prothrombin time).

Alternative Prothrombin Time and APTT Protocols

Coagulation Determinations: Prothrombin Times and APTT values are determined in HUMAN PLASMA with a STA instrument (Stago). BioPT is a special non-plasma clotting assay triggered with human tissue factor (Innovin). Possible binding to albumen or to lipid are assessed by comparing the BioPT effects in the presence/absence of 30 mg/mL human albumen (HSA) and 1 mg/mL phosphatidyl choline (PC). Inhibitors are delivered in 50% aqueous methanol vehicle.

APTT ASSAY

75 μL plasma Citrol *Baxter-Dade* Citrated Normal Human Plasma

25 μL test solution

75 μL Actin *Baxter-Dade* Activated Cephaloplastin incubate 2 min min. @ 37 °C

75 μl $CaCl_2$ (0.02 M)

PT ASSAY

75 μL plasma

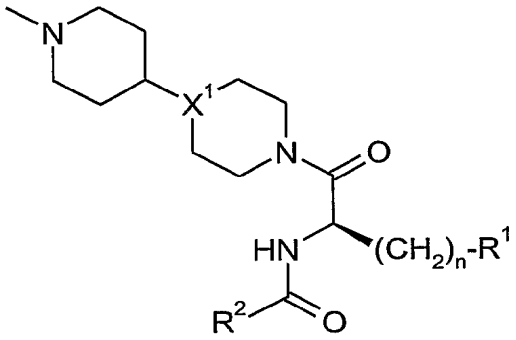
25 μL test solution

75 μL saline incubate 1 min. @ 37° C

75 μL Innovin *Baxter-Dade* Recombinant Human Tissue Factor

CLAIMS

1. A compound of formula (I)



(I)

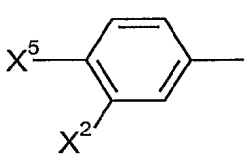
in which

X^1 represents CH or N;

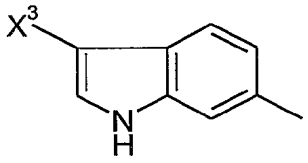
n is 1 or 2;

R^1 represents trifluoromethyl, COOH, $CONH_2$, SO_2NH_2, phenyl, pyridyl, C-linked imidazolyl (which may bear an N-(1-4C)alkyl substituent) or a (3-6C)cycloalkyl, oxa(4-6C)cycloalkyl, thia(4-6C)cycloalkyl or C-linked aza(4-6C)cycloalkyl group, which C-linked aza(4-6C)cycloalkyl group may bear an N-(1-4C)alkyl substituent; and

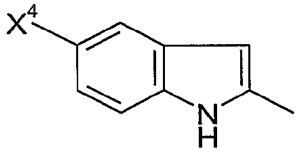
R^2 is selected from



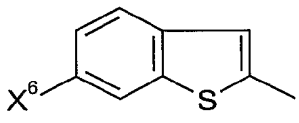
;



;



and



in which

X^2 represents a hydrogen atom, a halogen atom or an amino group;

X^3 represents a hydrogen atom, a methyl group, a fluorine atom, a chlorine atom or a bromine atom;

X^4 represents a hydrogen atom, a methyl group or a halogen atom;

X^5 represents a chlorine atom, a methoxy group or a methyl group; and

X^6 represents a hydrogen atom, a halogen atom or a methyl group;

or a pharmaceutically acceptable metabolically labile ester thereof, or a pharmaceutically acceptable salt thereof.

2. A compound as claimed in Claim 1, in which R^1 represents trifluoromethyl, COOH, $CONH_2$, phenyl, pyridyl, N-(1-4C)alkylimidazol-4-yl or a cyclopropyl, cyclohexyl, oxetanyl, tetrahydropyranyl, azetidinyl or piperidinyl group, which azetidinyl or piperidinyl group may bear an N-(1-4C)alkyl substituent.

3. A compound as claimed in Claim 2, in which R^1 represents trifluoromethyl, COOH, $CONH_2$, phenyl, pyrid-2-yl, pyrid-3-yl, pyrid-4-yl, N-methylimidazol-4-yl, cyclopropyl, cyclohexyl, tetrahydropyran-4-yl or an N-methylpiperidin-4-yl group.

4. A compound as claimed in any one of Claims 1 to 3, in which X^2 represents a hydrogen atom or a halogen atom.

5. A compound as claimed in Claim 4, in which

X^2 represents a hydrogen atom or a fluorine atom;

X^3 represents a hydrogen atom, a fluorine atom, a chlorine atom or a methyl group;

X^4 represents a chlorine atom;

X^5 represents a chlorine atom or a methoxy group; and

X^6 represents a chlorine atom.

6. A compound as claimed in Claim 5, in which R^2 is 4-chlorophenyl, 4-methoxyphenyl, 3-fluoro-4-methoxyphenyl, indol-6-yl, 3-methylindol-6-yl, 3-chloroindol-6-yl, 5-fluoroindol-2-yl, 5-chloroindol-2-yl or 6-chlorobenzo[b]thiophen-2-yl.

7. A compound as claimed in Claim 6, in which R^2 is 4-methoxyphenyl, indol-6-yl or 5-chloroindol-2-yl.

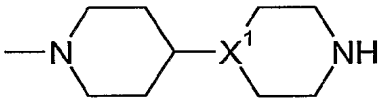
8. A compound as claimed in any one of Claims 1 to 7, in which X^1 represents CH.

9. A compound as claimed in any one of Claims 1 to 7, in which X^1 represents N.

10. A pharmaceutical composition, which comprises a compound as claimed in any one of Claims 1 to 9, together with a pharmaceutically acceptable diluent or carrier.

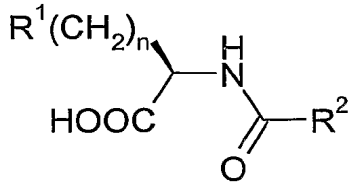
11. A process for preparing a compound as claimed in any one of Claims 1 to 9, which comprises

(a) reacting a compound of formula (II)



(II)

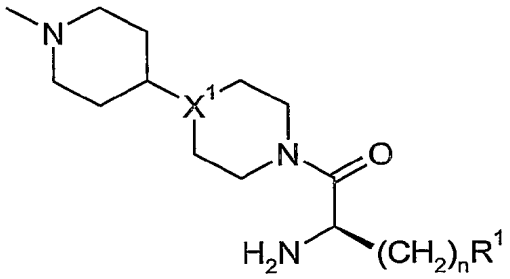
or a salt thereof, with a compound of formula (III)



(III)

or a reactive derivative thereof; or

(b) reacting a compound of formula (IV)



(IV)

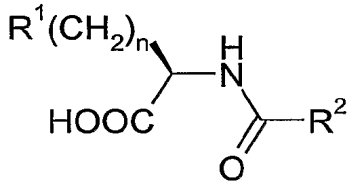
or a salt thereof, with a compound of formula (V)

(V)

or a reactive derivative thereof;

followed, if a pharmaceutically acceptable metabolically labile ester or a pharmaceutically acceptable salt is desired, by forming a pharmaceutically acceptable metabolically labile ester or salt.

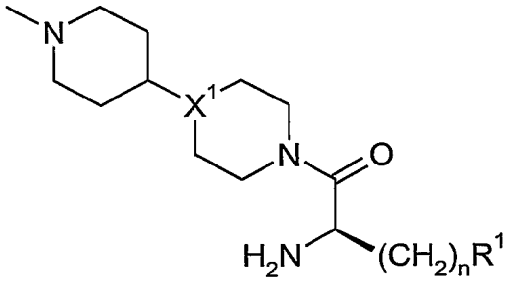
12. A compound of formula (III)



(III)

or a salt thereof, in which R^1 and R^2 are as defined in Claim 1.

13. A compound of formula (IV)



(IV)

or a salt thereof, in which X^1 and R^1 are as defined in Claim 1.

14. A compound as claimed in any one of Claims 1 to 9, for use in therapy.

15. Use of a compound as claimed in any one of Claims 1 to 9, for the manufacture of a medicament for the treatment of a thrombotic disorder.

16. A method of treating a thrombotic disorder in a subject requiring treatment, which comprises administering an effective amount of a compound as claimed in Claim 1.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/37595

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D401/12 C07D401/14 C07D401/04 C07D409/12 C07D409/14
C07D209/04 C07D333/52 A61K31/38 A61K31/404 A61K31/445

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 55154 A (BOEHRINGER INGELHEIM PHARMA ;EBERLEIN WOLFGANG (DE); HALLERMAYER G) 21 September 2000 (2000-09-21) page 32, last paragraph; claim 1	1-16
A	WO 01 10425 A (BOEHRINGER INGELHEIM PHARMA ;EBERLEIN WOLFGANG (DE); DOODS HENRI ()) 15 February 2001 (2001-02-15) the whole document	1-16
A	WO 99 11657 A (CREW ANDREW PHILIP AUSTIN ;JONES STUART DONALD (GB); MORGAN PHILLI) 11 March 1999 (1999-03-11) cited in the application the whole document	1-16

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 February 2003	04/03/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Samsam Bakhtiary, M

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 02/37595

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claim 16 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 02/37595

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0055154	A	21-09-2000	DE	19911039 A1	14-09-2000
			AU	4288000 A	04-10-2000
			CA	2361939 A1	21-09-2000
			WO	0055154 A1	21-09-2000
			EP	1163239 A1	19-12-2001
			JP	2002539208 T	19-11-2002
			US	6313097 B1	06-11-2001
WO 0110425	A	15-02-2001	DE	19937304 A1	15-03-2001
			AU	6992800 A	05-03-2001
			BG	106391 A	30-09-2002
			BR	0013009 A	30-04-2002
			CN	1370069 T	18-09-2002
			CZ	20020497 A3	12-06-2002
			WO	0110425 A2	15-02-2001
			EP	1207884 A2	29-05-2002
			HU	0202397 A2	28-10-2002
			NO	20020605 A	07-02-2002
			SK	1972002 A3	04-06-2002
			TR	200200359 T2	21-05-2002
WO 9911657	A	11-03-1999	AU	8875398 A	22-03-1999
			AU	8875798 A	22-03-1999
			EP	1012166 A1	28-06-2000
			EP	1009758 A1	21-06-2000
			WO	9911657 A1	11-03-1999
			WO	9911658 A1	11-03-1999
			US	6262069 B1	17-07-2001
			US	2002040144 A1	04-04-2002
			US	2002055522 A1	09-05-2002